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Feeding, swimming and respiration in barnacle larvae (Cirripedia: Thoracica)

Candeias, Adriana

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Feeding, Swimming and Respiration in Barnacle Larvae (Cirripedia: Thoracica)

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Adriana Tojinha Candeias

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School of Ocean Sciences
University of Wales – Bangor
Porthaethwy (Menai Bridge)
Ynys Môn
LL59 5AB

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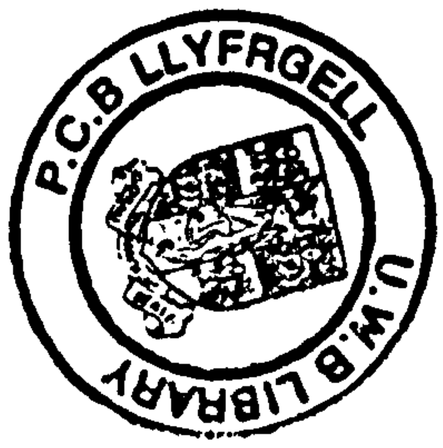

Dedico esta tese aos meus "progenitors",

Família Tojinha Candeias,

Manuel & Irene & Humberto.

A eles devo quem eu sou e o que consegui.
O seu amor e apoio incondicionais guiam-me sempre e
mesmo quando a sua presença fisica não é permanente, estão sempre comigo,
inspirando-me nas minhas batalhas, nas minhas escolhas, na minha liberdade e no
meu crescimento, como adulta e como ser humano.
O seu papel e acção na sociedade ensinam-me a respeitar e a ser respeitada, a amar e
cuidar, a ser honesta e forte, a estar tranquila e alegre, a rir e a não ter medo,
mesmo quando choro.
A vida é uma coisa muito bonita e neles, eu vejo um Mundo melhor.

"Amo-vos mais que ao Sol e à Água, meus lindos cravos vermelhos".

I dedicate this project to my "progenitors",
Family Tojinha Candeias,
Manuel & Irene & Humberto.

To them I owe who I am and all that I have achieved.
Their unconditional love and support have guided me always and although life has brought me apart from their closer contact, they have always been there for me; they have inspired my battles, my choices, my freedom and my growth as an adult and as a human being.
Their role and attitude in the society teach me to be respectful and respected by others, to love and care, to be honest and strong, to be gentle and joyful, to laugh and not to fear, even when crying.
Life is a beautiful thing and in them, I see a better World.

"Amo-vos mais que ao Sol e à Água, meus lindos cravos vermelhos".

FOREWORD

© João Mariano

"A experiência acumulada, cujo exame e conhecimento são essenciais – embora abranja uma infinidade de experiências individuais e contenha sempre ensinamentos e novidades a receber da experiência individual de cada militante –, é sempre uma experiência colectiva."

(The accumulated experience, of which examination and acquisition are essential – though integrating an infinite number of individual experiences and always incorporating teachings and novelties from the individual experience of each militant –, it is always a collective experience.)

Álvaro Cunhal (1913-2005)
in "O Partido com Paredes de Vidro", Edições Avante, 1985.

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SUMMARY

For planktotrophic larvae, the availability of food is one of the major factors that control growth and development. In the present study, feeding and starvation were investigated within the scope of survival, swimming and respiration rates of Cirripedia larvae.

Adults of *Pollicipes pollicipes* (Pedunculata: Scalpellidae) and *Elminius modestus* (Sessilia: Balanidae) were collected from the Iberian Peninsula and western UK coast, respectively, and a series of laboratory experiments conducted using a novel rotating wheel immersed in a temperature controlled tank. Observations were made on larval growth, gut size, mobility and oxygen consumption in response to both the supply of food in the form of controlled algal sources, as well as starvation conditions. Resulting data were analysed and revealed notable trends in the relationship between the different functional outputs at different stages of development, up to and including the cyprid.

Mono- and mixed algal cultures were tested, confirming that both barnacle species have geographical related dietary preferences. Size of algal cells was only one of the factors associated with feeding rates, while volume densities and quality of the supplied phytoplankton proved of substantial importance throughout the whole study.

Under starvation conditions, swimming performance did not deteriorate during 28 hours after hatching, indicating that *E. modestus* nauplius II carry enough yolk reserves to proceed the search for food. Nonetheless, oxygen consumption reduced after 8 hours indicating that larvae are able to adjust their metabolism as an energy conservation strategy. This would account for the oceanic distribution of spawned larvae even under conditions of impoverished plankton supply. Increase in oxygen consumption in earlier larval stages is associated with high energy expenditure of swimming and capture of food, while during the metanauplii, stable weight specific respiration rate accompanied by reduced swimming speeds suggests an increase in non-swimming related metabolic activity, possibly reflecting a radical physiological and functional shift at this stage. The first demonstration of specific dynamic action in barnacle larvae is discussed.

The details provided on specific feeding rates and development, algal preference, physiological processes and swimming behaviour of barnacle larvae, contribute to the understanding of the effect of barnacles on the phytoplankton while part of the meroplankton communities.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I General Introduction

I.1. Meroplankton and Cirripedia

Meroplankton (meros μέρος = portion, share + planao πλανάω = lead astray, mislead) classifies the organisms which form part of the zooplankton community temporarily during their life cycle, mainly for dispersal purposes. Species of molluscs, cirripeds, decapods, echinoderms, polychaetes, ascidians and fish have "wandering" larvae, the development stages being free in the water column, taking advantage of the availability of nutrients until they settle to become part of the benthos (Raymont 1983).

On a temporal scale, these organisms can be predominant and have a significant role on the diversity of the marine plankton community, serving as important ecological indicators. In addition, concern for management of the marine environment is growing as the search for natural resources continues to increase (Souvorov 1999). Hence, studying meroplankton as part of the food web is key to our understanding of the sea and coastal productivity.

Adult forms of Cirripedia have evolved to be sessile, with most typical crustacean structures being modified or non-existent. Barnacles, as they are commonly called, assume different morphology as a consequence of their adaptation to diverse environments and to escape predation and competition (Foster 1987). Parasitic forms (particularly orders Rhizocephala and Ascothoracica) specialise in the host organism, generally lacking appendages and digestive tract and developing a foot-like absorptive process from the peduncle (e.g. *Sacculina carcini*). According to Darwin, free living barnacles can be classified as stalked (with a muscular, flexible stalk that is attached to the substrata, a capitulum and the carapace that surrounds the mantle; Order Pedunculata) and sessile (stalkless, without a peduncle; Order Sessile) (Ruppert & Barnes 1994). This classification was considered artificial after deduction that the asymmetrical (Verrucomorpha) and symmetrical (Balanomorpha) sessile barnacles are polyphyletic (Newman 1987).

Barnacles can be found on temperate rocky shores (e.g. *Chthamalus stellatus*, *Verruca stroemia*), drifting on the surface of oceans attached to floating objects (e.g. *Lepas anatifera, Lepas fascicularis*), hydrothermal vents (e.g. *Neolepas sp.*), on tropical mangrove roots (e.g. *Balanus eburneus*) or commensally on other marine organisms (e.g. *Acasta spongites*, *Megatrema anglicum*, *Solidobalanus fallax*, *Mya arenaria*). Despite being highly diversified, all barnacles share the cypris, the lecithotrophic last larval stage, characterised by a bivalved-shape carapace, propulsive thoracic appendages and a developed nervous system (Harrison & Sandeman 1999, Blomsterberg 2004), and in all planktotrophic thoracican barnacles, the cyprid is preceded by six naupliar stages.

Each year, barnacle settlement causes dramatic financial losses to the fishing, transport, aquaculture and leisure industries, as they are responsible for ship fouling and degradation of immersed installations at sea. For this reason, manufacturers have encouraged research on bioadhesion and larval settlement cues in order to find resistant compounds to biofouling (Crisp 1974a, Maki *et al.* 1990, Sasikumar *et al.* 1994, Yule 1994, Berglin & Gatenholm 2003, Khandeparker *et al.* 2005).

On the other hand, Cirripedia play an important role both as meroplankton, while larvae, and as part of the benthic community, while adults. At community scale, different species of barnacles have different breeding patterns, and are thus present in the water column all year round, often their abundance second only to copepods (Carrie 1964 and Eriksson 1973 *in* Raymont 1983, Muxagata *et al.* 2004). Once settled, barnacles act as bioconstructors, modifying the substrate and habitat structure, thereby influencing the composition of biota and promoting species diversity (Cocito 2004). Researchers emphasize the contribution of barnacle larvae to the carbon flux in pelagic waters (Lang & Ackenhusen-Johns 1981, Moscatello *et al.* 2004, Muxagata *et al.* 2004) and modern biological oceanography should consider this planktonic group as important research object.

I.2. *POLLICIPES POLLICIPES* – THE GOOSENECK BARNACLE

Pollicipes pollicipes (Gmelin 1790) (Pedunculata: Scalpellidae) is a stalked barnacle, characterised by a flexible muscular peduncle fixed to the substrata. It is distributed along the Atlantic rocky coasts of south France, Spain, Portugal and north-west Africa (Stubbings 1967, Cruz 1993, Molares & Freire 2003), where it forms dense aggregated populations, usually associated with the *Mytilus galloprovincialis* or *M. edulis* community.

In the Iberian Peninsula, the "percebe" is considered an exquisite delicacy, representing an important or exclusive income for local fishermen, where prices can reach €80/kg in first-sale auctions (Molares & Freire 2003). Over-exploitation and stock depletion (Bernard 1988, Cardoso & Yule 1995) has attracted attention for research. *In vitro* settlement experiments of this species have rarely been successful (Goldberg 1984) with relatively more effort dedicated to conservation and recovery of the extant populations. The importance of this trade in Galicia (NW Spain) has justified the implementation of a geographic information-based system for population co-management between organizations of fishermen and the fisheries authorities through territorial user rights for fishing (Molares & Freire 2003). The highly exposed shores where *P. pollicipes* is found add to the economic value of this animal. The violent waves make access to the populations extremely difficult and dangerous, providing restricted availability to both fishermen and researchers alike.

Knowledge of this species has been gained particularly from research in the fields of fisheries and product quality (Molares *et al.* 1987, Molares & Freire 2003), distribution and growth (Coelho 1991, Cruz 1993), life history and recruitment (Molares 1994, Macho *et al.* 2005, Vazquez *et al.* 2005) and gametogenesis and reproduction (Molares *et al.* 1994, Cardoso & Yule 1995, Cruz & Araújo 1999).

Several authors have previously ascribed *P. cornucopia* (Leach 1824) to adult and larval features of *P. pollicipes* (Darwin 1851, Azevedo & Corral 1982, Molares *et al.* 1987, Molares 1994), but more recently the scientific name has been taken to be that of the original described by Gmelin (1780) (e.g. Molares & Freire 2003).

Currently, there are only three species assigned to the genus *Pollicipes* and the lack of literature on *P. pollicipes* is often compensated by comparison with *P. polymerus*, its relative from the western coast of the north of the American continent.

Studies on *P. polymerus* include feeding and physiology (Barnes & Reese 1959), reproduction and larval development (Lewis 1975a, 1975b), and ecology, distribution and fisheries (Barnes & Reese 1960, Bernard 1988, Hoffman 1989, Lessard *et al.* 2003). *P. elegans,* from the west coasts of Mexico, Peru, and Chile, has been comparatively less studied (Kameya & Zeballos 1988, Van Zyoc 1994).

Thus, it is only relatively recently that the larval ecology and physiology of *P. pollicipes* has started to be addressed by researchers. Specific interest is now being directed to post-hatching and pre-settlement larval transport and the abiotic factors conditioning recruitment (Molares, pers. comm.). The breeding season of *P. pollicipes* is from March to September (Cardoso & Yule 1995, Molares 1994), limiting the access to larvae for laboratory experimentation. Nevertheless, this should not discourage biologists from undertaking further investigation of the physiological processes, biochemistry and genetics, and, considering the economic importance of the "percebe", opportunities should be sought for scientific funding in this field.

I.3. *ELMINIUS MODESTUS* – THE SMALL BARNACLE

Elminius modestus Darwin 1854 (Sessilia: Balanidae), originally from Australasia, was first reported in the early 1940's in nearby Chichester Harbour Key (Stubbings 1967). Initial colonization, thought to have been by shipping (on ships' hulls, flying boats or transport of pelagic larvae in ballast water), was extensive, and it spread rapidly along the coast. This barnacle has been found all around the British mainland coast (Crisp 1958, Collins 1959), in Shetland (Hiscock *et al.* 1978) and the Outer Hebrides (Howson *et al.* 1994). It is distributed on the Atlantic coasts of Europe from Gilbraltar to Germany (Barnes & Barnes 1966), where the low temperature seems to restrict northwards spread of this species.

E. modestus produces several broods per year, competing in the northern waters with *Semibalanus balanoides*, which only produces one brood per year and earlier in the season. It tolerates lower temperatures than *Chthamalus spp.*, with which it competes in southern Europe. It also has a higher tolerance to salinity, inhabiting substrate where native species do not survive. It can settle both high up the shore and in the sublittoral zone and it tolerates higher temperatures than the endemic *Balanus*.

Consequently, *E. modestus* not only competes with Atlantic native barnacles, but also has colonised some sheltered habitats not previously inhabited by them, where it also withstands high turbidity. Although *E. modestus* may have displaced *B. improvisus* from estuaries and bays (Crisp 1958, Hayward & Ryland 1990, Lawson *et al.* 2004), it seems to have had little effect on the structure of exposed rocky shore communities, where native species (e.g. *S. balanoides*, *C. montagui*, *C. stellatus*) grow better. Barnett, Edwards & Crisp (1979) have argued that settlement is greater on conspecific species which leads to a greater intraspecific competition, rather than making it more severe between overlapping species. This encourages the settlement of the same species and exclude others from its territory (Barnett & Crisp 1979). Nevertheless, different recruitment patterns have allowed the coexistence of a mixed barnacle fauna, where seldom adults are seen to settle on the top of the carapace of other species.

While larvae of this species have been investigated in topics such as breeding and reproduction (Crisp & Patel 1961, O'Riordan & Murphy 2000), feeding and energetics (Barnes 1971, Harms 1987, Stone 1988), rearing and cultivation (Wisely 1959, Harms 1988), settlement (Ros 1989, Yule 1994, Neal *et al.* 1996, Ross 2001), and pollution (Mortlock *et al.* 1984, Billingurst *et al.* 2001, Rainbow & Wang 2001, Crowe *et al.* 2004), studies on physiological behaviour declined after the 1980's and new technologies and methodologies are yet to be applied to the study of this group of animals.

Because of its wide geographic distribution and its occurrence in a diverse range of habitats, study of *E. modestus* enables understanding of the adaptation of barnacles to different conditions. In addition, that it is widely available and produces larvae throughout the year assisted the present study, particularly when additional material was required for repeat experiments.

I.4. BARNACLE LARVAL DEVELOPMENT

I-4.1 Identification

Thoracican cirripedes develop through a sequence of six naupliar stages followed by a presettlement metamorphosis to a single cyprid stage. During the naupliar development, the general form and shape remain unchanged. The naupliar limbs act in swimming, food collection and ingestion in the same general manner during all stages and most parts of the naupliar body retain the same proportion. The major external changes during development are the increase in size of the thoraco-abdominal process, the development of several ventral spines and the increase in the number and types of setae of the naupliar limbs. In the case of the present study, all naupliar stages are planktotrophic, with the exception of stage I nauplius (NI), which do not possess developed mouth and gut and, like the cyprids, are lecithotrophic.

The labels used to distinguish the naupliar stages through out the current work were: stage I nauplius (NI), stage II nauplius (NII), stage III nauplius (NIII), stage IV nauplius (NIV), stage V nauplius (NV) and stage VI nauplius (NVI).

It is possible to identify the pedunculate larvae depending on total length, shield width, shape of the labrum, setation formula, and presence of posterior shield spine (Figure I-1). With the development of light and scanning electron microscopy, differentiation between species has become more detailed and in addition to general morphology, shape of the labrum and setation formula have been described for several species of cirripedia (Knight-Jones & Waugh 1949, Rainbow & Walker 1976, Kugele & Yule 1996, Lee *et al.* 2000, Rybakov *et al.* 2002, Yan & Chan 2001, Yan 2003). This enabled a better understanding of the larval structures and their functionality (Walker *et al* 1987, Harrison & Sandeman 1999).

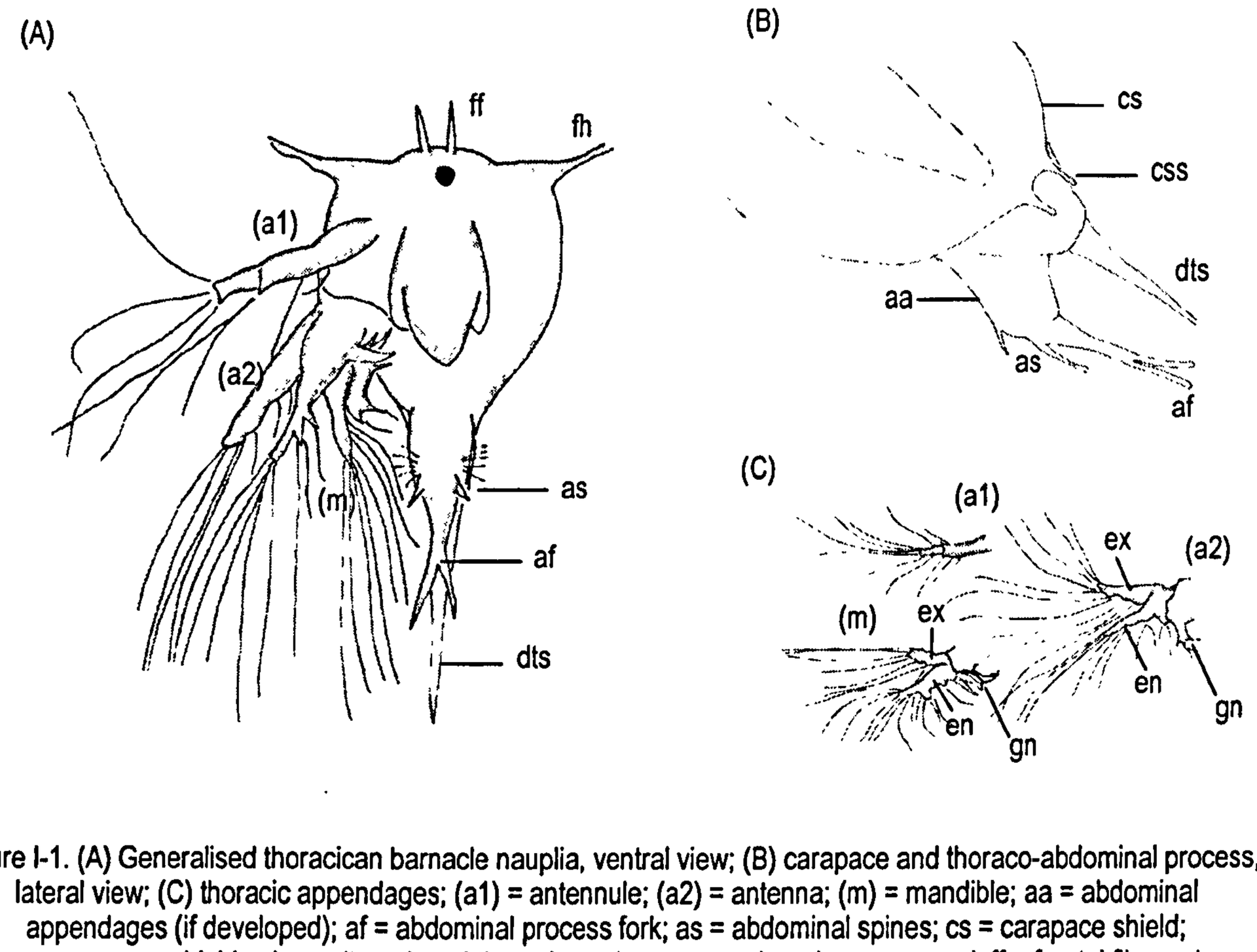


Figure I-1. (A) Generalised thoracican barnacle nauplia, ventral view; (B) carapace and thoraco-abdominal process, lateral view; (C) thoracic appendages; (a1) = antennule; (a2) = antenna; (m) = mandible; aa = abdominal appendages (if developed); af = abdominal process fork; as = abdominal spines; cs = carapace shield; css = carapace shield spines; dts = dorsal-thoracico spine; en = endopod; ex = exopod; ff = frontal filaments; fh = fronto-lateral horns; gn = gnathobase; la = labrum; ne = naupliar eye (after Knight-Jones & Waugh 1943, Walker *et al.* 1987, Anderson 1994, and photographs taken during present work)

Knight-Jones & Waugh (1949) described the general characters of larval development of *E. modestus* including the setation formula for all naupliar stages, given by the Bassidale (1936) formulae, and Kugele & Yule (1996) described setation of *P. pollicipes* naupliar stages, using Newman (1965) notations. Though essential for identifying larvae at taxonomic level after dissection, setation formulae proved to be extremely difficult to apply on the staging of active larvae. Table I-1 and Table I-2 provide a key for identification of *E. modestus* and *P. pollicipes* nauplii, with particular emphasis on recognizable features of fast swimming animals.

Table I-1. Dichotomous key for identification of naupliar stages of *Elminius modestus* (after Knight-Jones & Waugh 1959).

- Fronto-lateral horns pointing obliquely backwards	**NI**	Short caudal spine and abdominal process; limb setae unfeathered
- Fronto-lateral horns point in a lateral or antero-lateral direction		
- Posterior carapace border inexistent or difficult to distinguish		Single pair of large spines on the latero-ventral surface of the abdomen
- Long dorsal-thoracic (caudal) spine and shorter, narrow abdominal process	**NII**	Prongs of the forked abdominal process are proportionally longer than in other stages.
- Dorsal-thoracic spine (caudal) and abdominal process are of the same length	**NIII**	Abdominal process is slightly swollen and body stouter build than NII.
- Carapace shield posterior border protrudes and bears a pair of carapace spines		Swollen abdominal process; two pairs of spines on the latero-ventral surface of the abdomen.
- Posterior pair of proeminent spines on the latero-ventral surface of the abdomen is widely separated and larger than anterior one	**NIV**	Easily distinguished carapace fold, rounded carapace border.
- Spines on the latero-ventral surface of the abdomen are close together and similar in size		Internal rudiments of the abdominal appendages can be distinguished.
- Six pairs of abdominal appendages are rudimental,	**NV**	Spines are very small and aligned at the same level of the ventral surface of the abdomen.
- Six pairs of abdominal appendages are prominent	**NVI**	Ventral surface of the abdomen bears six pairs of small spines, segmentally arranged; three black eye spots can be occasionally recognised.

Table I-2. Dichotomous key for identification of *Pollicipes pollicipes* naupliar stages (after Molares 1994, Kugele & Yule 1996)

- Fronto-lateral horns pointing obliquely backwards	**NI**	Short caudal spine and abdominal process; limb setae unfeathered; no spines on ventral thoracic process
- Fronto-lateral horns point in a lateral or antero-lateral direction		
- Posterior carapace border inexistent or difficult to distinguish		
- Longer and relatively thinner fronto-lateral horns	**NII**	Prongs of the forked abdominal process are proportionally longer than in other stages. Fronto-lateral horns slightly antero-lateral orientated
- Shorter front-lateral horns with brush like extremities	**NIII**	Abdominal process is swollen and body stouter build than NII. Fronto-lateral horns are perpendicular to larval longitudinal axis.
- Carapace shield posterior border protrudes		Gradual swollen of abdominal process; two pairs of spines on the latero-ventral surface of the abdomen.
- Absence of six pairs of spines aligned in two segments in ventral surface of abdomen		
- Two pairs of proeminent spines on the latero-ventral surface of the abdomen		
- Two pairs of spines are very close together	**NIV**	Easily distinguished carapace fold, lateral outline of carapace is perpendicular to anterior axis.
- Two pairs of spines are widely separated and rudiments of abdominal appendages can be distinguished	**NV**	Wider body, hexagonal carapace shield outline
- Six pairs of abdominal appendages are prominent, aligned longitudinally along ventral surface of abdomen	**NVI**	Carapace shield very convex; three black eye spots can be occasionally recognised.

I-4.2 Ecology and metabolism of barnacle larvae

The larvae investigated in the present study are planktotrophic, i.e. swim and feed in the plankton (Thorson 1950), although the first and the last stages, NI and cyprid, do not feed and rely on the reserves placed in the egg during oogenesis or accumulated through out the larval development, respectively. They are pelagic (Mileikovsky 1971), passing through an indirect free-living development in the water column before they reach an appropriate substratum where they metamorphose into juveniles.

Wide dispersal to diverse habitats, with potential increase in geographical range and population size, is one of the functions of pelagic larvae and it might have been the reason why this larval form evolved in the life cycle of benthic organisms (Wray 1995). Larval feeding allows exploitation of different food sources early in the life cycle, increasing brood sizes and recruitment success. According to Scheltema's classification (1989 *in* Levin & Bridges 1995), *P. pollicipes* and *E. modestus* larvae are actaeplanic (actaeos = coastal, planos = wanderer), i.e. coastal larvae with planktonic periods of 1 week to <2 months. During this period, these larvae have to balance dispersal potential, investment of energy, potential successful recruitment and avoidance of mortality sources (predators and physical constraints) (Levin & Bridges 1995).

Several authors have discussed and presented different opinions about swimming and feeding mechanisms of thoracican nauplii (Walker *et al.* 1987), but it is generally accepted that these processes are intrinsically connected as the three pairs of limbs participate differentially, in both. A chemosensory response appears to be the main driver for feeding rates, as shown by experimentation of feeding on food particles (dissolved amino acids and sugars in water) (Yule 1982, Anderson 1994). Also, the ambient temperature is a principal factor on limb beat rate and style of swimming (Yule 1984), which also affects the transport of particles inside the digestive tract and therefore the ingestion rate.

Zooplankton suffer high mortality due to predation or lack of food, thus surviving larvae require high performance and fitness during the development stages. Larvae inhabit environments where their nutritional requirements are satisfied, although they will adapt to variable conditions to some extent. Establishment of stress factors

leads to survival cues, i.e. the definition of resistance levels or sensitivity to a certain lacking nutrient helps to characterise the adaptation of the larvae to the environment.

Knowledge of feeding and capture of food is essential for understanding metabolism of planktotrophic larvae. At stage NI, *E.modestus* and *P. pollicipes* are lecithotrophic, possessing simple morphological structures and being released from the adults at a very low reproductive cost. On the other hand, the cypris is also lecithotrophic, and it spends the accumulated lipid energy reserves for swimming and exploration, thus settlement has to be successful before those reserves are utilised (Holland 1987, Thiyagarajan *et al.* 2002). During development, larvae increase their physiological complexity and chances of successful settlement, therefore metabolic requirements are expected to be different. Respiration rate has been used as an indirect measure of metabolism but its application to zooplankton has been restricted. Advances in accurate instruments that can detect small variations in oxygen concentration have allowed respiration assessment of specific groups of zooplankton (Sell 2000, Lenz *et al.* 2005 (Copepoda), St-Amand 1999 (Decapoda), Wendt 2000 (Bryozoa), Parra & Yufera 2001, del Carmen Alvarez & Fuiman 2005 (fish)), although much remains to be investigated in the area of energy budget of planktonic animals.

It is important to take into consideration the diversity of factors affecting larval development and the differential effect those factors may have within the same larval stage. In addition, because larval processes may evolve in response to adult diet, size, reproduction and growth (Holland 1994), it is critical to integrate studies of early life stages with those of juveniles and adults and synthesise in a broader sense the life history and ecology of marine animals.

I.5. SCOPE AND RATIONALE

There is a growing awareness of the interplay between all the factors that influence communities. Examination of a single process or group of organisms seems futile when considering the bottom-up and top-down regulating factors, and only multidisciplinary research projects are believed to elucidate the intricate matrix of ecosystem structure (Pomeroy 2004).

Nevertheless, as reductionist as it may seem, marine ecology can only benefit from the added knowledge of taxonomists and physiologists. By dedicating time and effort to understand form-function processes, species requirements and interactions, they have the opportunity to grasp the diversity within and between organisms, which consequently substantiates the idea of the communities' complexity.

For the reasons presented above, Cirripedia and in particularly barnacle larvae should not be disregarded under the scope of marine studies. As part of a wide network of organisms, barnacle population dynamics should be discussed along with the processes that control larval development and recruitment. However, undertaking analyses of metabolism and behaviour of zooplankton *in situ* is extremely difficult. Naturally, the majority of experimentation has been carried out under laboratory conditions. This may be done either by attempting to reproduce natural conditions in the laboratory, or perhaps more realistically, trying to investigate the response to each factor separately.

The present study attempts to answer some detailed questions concerning the feeding, swimming, respiration and response to starvation of two barnacle species. Without being tempted to extrapolate the results directly to the natural environment, the findings of the following chapters may be applied under the conditions described. As such, they can be considered as fragments of the enormous jig-saw of understanding the oceans energetics.

This first chapter, introduces the biology of thoracican barnacles and explains the importance of Cirripedia research in relation to the study of marine ecosystems. It summaries previous works, distinguishes both species investigated in this thesis and justifies the need for further experimentation on metabolism of this group of animals.

The second chapter, General Methodology explains techniques for adult sampling, larval and algal rearing and larval size measurements, employed throughout the entire project and common to several experiments. It also describes a rotation device designed especially for experimentation on zooplankton. Particular protocols are further explained in each respective following chapters.

The third chapter is the first of experimentation and concentrates on feeding of *Pollicipes pollicipes*. It compares ingestion rates on different algal monocultures and analyses efficiency of diets on larval development and survival rates. It explores phytoplanktonic feeding of this barnacle larvae and attempts to place these results within the ecology of this species.

The fourth chapter investigates the feeding behaviour of *Elminius modestus* nauplii II when offered two suitable algae as food, at different concentrations and proportions. Hypotheses of selective feeding are explored while experimental conditions are discussed within the scope of mathematical calculations.

Chapter five looks into the swimming of *E. modestus* larvae and *P. pollicipes* metanauplii, calculating larval speeds along the development stages. It investigates the effect of starvation in larval mobility and describes behavioural changes between larval moults. Change of shape is analysed under the larval functionality and water physics are considered in ecological interpretations of larval development stages.

The sixth chapter is the last one of results and it determine oxygen consumption of *E. modestus* larvae in relation to food presence and diet type. Starvation of nauplii I is also examined and respiration rate is evaluated in relation to dry weight, i.e. body mass increment through out development.

The last chapter is the General Discussion, which brings together the results obtained in the previous chapters. The changes in feeding, behaviour and metabolism during larval development, particularly in relation to starvation, are interpreted within the scope of physiological energetics and ecological adaptations occurring during the planktonic stages of barnacles.

II General Methodology

II.1. SAMPLING SITES AND ANIMAL COLLECTION

II-1.1 Iberian Peninsula

Cape São Vicente, on the Southwest Coast of Portugal, is the most southwesterly point in Europea. Markedly seasonal, the coastal waters are characterised by a complex summer upwelling of cold subsurface water, which is yet to be fully understood (Relvas & Barton, 2002, 2005). Experiencing the full force of Atlantic waves and fast coastal water currents, the dramatic landscape is one as tall and rough cliffs intersects with sandy beaches. The high primary production of the area makes it ideal for the development and settlement of *P. pollicipes*. The Galician coast of Spain shares many hydrographic and topographical characteristics with the southwest coast of the Algarve, hence *P. Pollicipes* were also collected from there (Figure II-2).

In Portugal, animals were collected at low tide, from the rocky shore of the Natural Park of Sudoeste Alentejano e Costa Vicentina (Fortaleza, Beliche, Tonel, Castelejo, Zavial, Almograve), and further north, at Peniche. In Galicia, animals were collected from Illa Ons, Illa Onsa, and Cape Home in Ria de Vigo and also from Cabanas (Rias Altas, A Coruña).

Adults were cleaned of adherent algae and benthic animals, such as mussels and polychaetes, and transported to the laboratories inside a thermo-cooling system, within 3 hours of collection. In experiments run in the School of Ocean Sciences, Menai Bridge, UK, (development and survival of *P. pollicipes*), animals were transported from Portugal and larval cultures set up within 36 hours of collection.

II-1.2 The Menai Strait

The Menai Strait was designated as a Special Area of Conservation (Menai Strait and Conwy Bay SAC), in December 2004, under the EC 'Habitats' Regulations, 1994. This seawater channel separates the island of Anglesey from the UK mainland in North Wales and has been well studied. Today, much is known about the planktonic, neritic, and benthic communities along with its major physical and oceanographic processes. The Menai Strait has always been place of intense human activity, constantly crossed by cargo vessels and pleasure craft used for leisure, trade, exploitation of sand and more recently mussel and oyster cultivation.

E. modestus have colonized Northwestern Europe since 1940, transported form its native Australasia on ships' hulls, flying boats or in ballast water (Barnes & Barnes 1960). In Menai Bridge it now forms mixed communities along with *Semibalanus balanoides* and *Balanus crenatus*, occupying the full extent of the intertidal zone. St. George's Pier is an easy sampling spot for *Elminius modestus*, within walking distance of the School of Ocean Sciences in Menai Bridge and offering a year round supply of adults carrying egg masses. *E. modestus* was easily identified at the pier pilings and could be readily scraped, individually, from the substratum (Figure II-2).

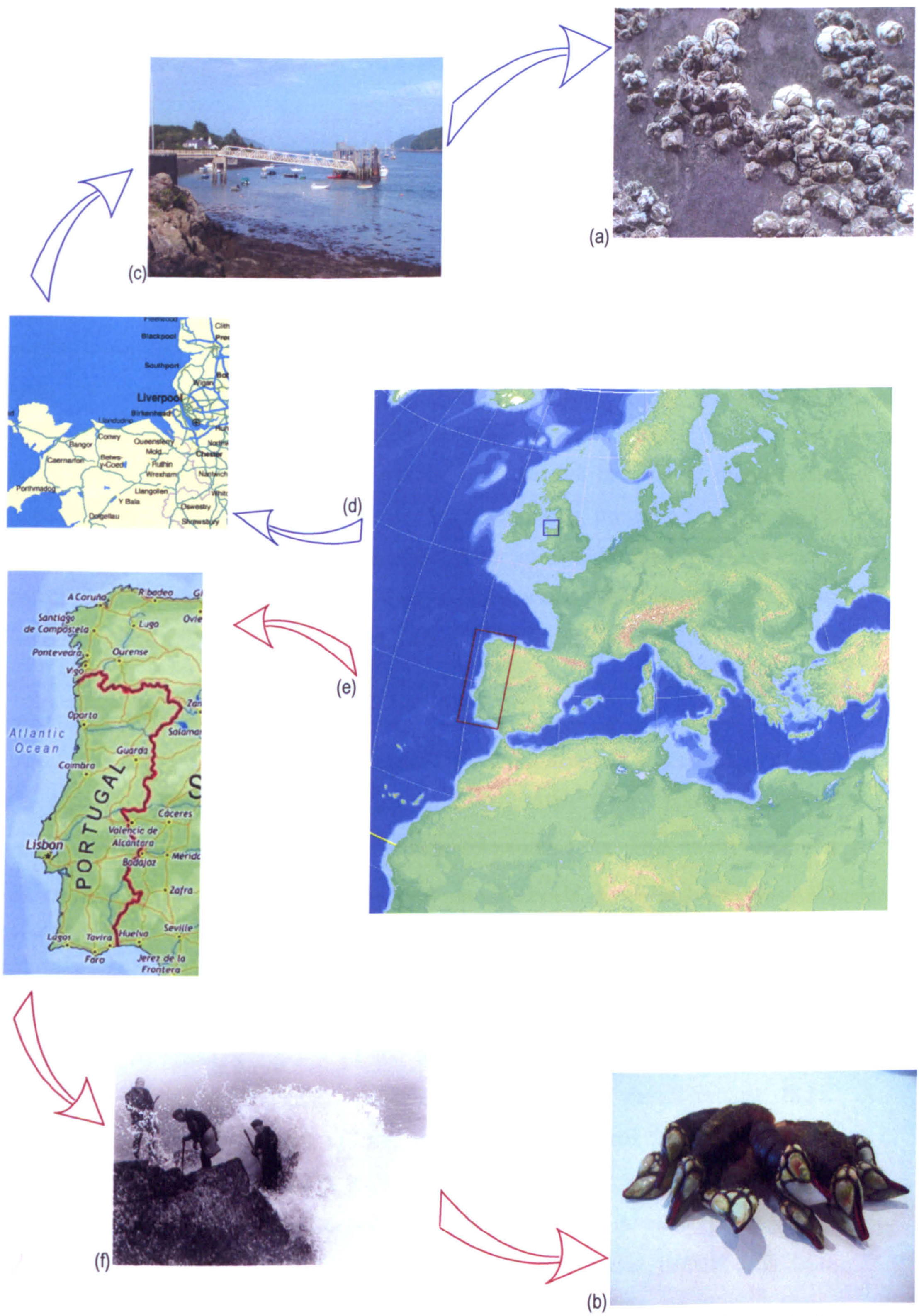


Figure II-2. Sampling sites for collection of *Elminius modestus* (a) and *Pollicipes pollicipes* (b), from the pier (c) in the Menai Strait, North Wales (d) and Galicia and Portugal (e), respectively. North Wales Maps Reproduced from Ordnance Survey mp data by permission of Ordnance Survey, © Crown copyright, Map of Portugal by Tourizm Maps © 2003 and (f) photograph of fisherman ("percebeiro") in the Portuguese southwest coast kindly provided by © João Mariano.

II.2. LARVAL REARING

II-2.1 *Pollicipes pollicipes*

Cultivation of barnacle larvae is relatively straight-forward (Moyse 1960, Tighe-Ford *et al.* 1970, Yule 1982). However *Pollicipes pollicipes* require more attention than many species (Kugele & Yule 1996) to avoid high mortality rates, which limits the success of obtaining later development stages. Elevated temperatures coupled with high algal and larval concentrations and consequently high levels of faecal pellets, contribute to proliferation of Protozoa and bacteria in much higher densities than in the wild. Within the laboratory, this presents a challenge; in comparison to the natural environment, laboratory cultivation tends to be at higher animal densities. Furthermore, collection and transportation of *P. pollicipes* from the Iberian Peninsula to the UK was not frequent thus, considerable attention was given to the cleanliness of cultures and effort was made to remove molds, accumulated faecal pellets and dead algae.

After collection from the wild, adults were transferred into tanks of filtered (0.2 μm) and UV sterilised seawater and fed with early larval stages of *Artemia*. Allowing the natural release of hatched nauplii from these adults assured a continuous supply of larvae but seldom in sufficient quantities required for experimentation. Consequently, egg sacs were removed from the mantle cavity of several adults on arrival to the laboratory. Each egg sac was placed in 100 mL filtered aerated sea water in volumetric flasks and kept under constant strong aeration to stimulate the mechanical rupture of the mature egg membrane. Isolated ovisacs released living NI, which quickly moulted to NII during the following 78 hr. Nevertheless, most larvae used for the cultures were obtained in the four hours immediately after dissection of the adult. Actively swimming larvae were transferred to 3000 mL jars containing 100 cells mL^{-1} of *Isochrysis galbana* (Galicia) or *Rhinomonas reticulata* (Menai Bridge) and stocked at a larval density less than 3 individuals mL^{-1}. The abundance of larvae was determined, by sampling 3 times 3 mL of the larval initial culture and determining the final total number of animals.

Generally, *P. pollicipes* cultures were maintained in clean UV filtered seawater, changed every 2-3 days and the larvae fed with algal suspension to a final concentration of 100 cells μL^{-1}. Culture vessels were disinfected with a 1% solution of sodium hypochlorite and carefully rinsed with hot tap water to remove any toxic residues. Larvae were gently sieved through an 80-µm nylon plankton mesh, which was placed inside a glass cylindrical container full of water to avoid mechanical damage. Larvae were carefully handled, in large volumes of water and low densities, to produce successful cultures. Naupliar stage, larval abundance, level of food in gut, swimming activity, limb cleanliness and general larval healthiness were assessed each time the cultures were cleaned.

II-2.2 *Elminius modestus*

Adults collected from the pier were transferred to the laboratory and larval release was induced by gently breaking the adults carapace in a bucket (see Yule, 1982). The shattered adult remains were immersed in filtered seawater and left to stand for release of hatched nauplii. It was found that when this mixture was sieved through 1000 µm and 250 µm meshes, larvae were obtained more quickly and abundantly. During this process, larger particles, including macroscopic algae, shells, sand and zooplanktonic predators were removed. The resulting suspension was transferred to opaque plastic trays and semi-covered with black lids, reducing the illumination. *E. modestus* NI were attracted to a light source using a fibre optic light guide and pipetted into UV radiated 0.2 µm-filtered seawater volumetric flasks.

The nauplii were transferred into 3 L jars, with *Rhinomonas reticulata* suspension (final density of 100 cells μL^{-1}), at an abundance of 1-3 animals mL^{-1}.

Every 2-3 days, the water was changed by gently sieving the larvae through a 80 µm mesh, in the same manner as described for *P. pollicipes*. Cultures were observed under a binocular stereo-microscope, moulds and dead animals were thoroughly pipetted out, and larval numbers, stage and general healthiness assessed for in each jar.

II.3. ALGAL REARING

The algal cultures used were (width / length ranges given in brackets):

Bacillariophyta (diatoms)

- *Chaetoceros gracilis* Shütt (7 / 80 µm)
- *Skeletonema costatum* Greville 1965 (2-21 µm / 2-61 µm)
- *Phaeodactylum tricornutum* Bohlin 1897 (3-8 µm / 25-35 µm)

Haptophyta (flagellate phase)

- *Isochrysis galbana* Parke 1949 (5 / 5 µm)

Chrysophyta (golden brown algae)

- *Pavlova lutheri* Green 1975 (4 / 10 µm)

Prasinophyta (green flagellates)

- *Tetraselmis chui* Stein 1878 (4 / 25 µm)

Chryptophycea (cryptomonad flagellates)

- *Rhinomonas reticulata* (Lucas) Novarino 1990 (3-4 / 6-8 µm)

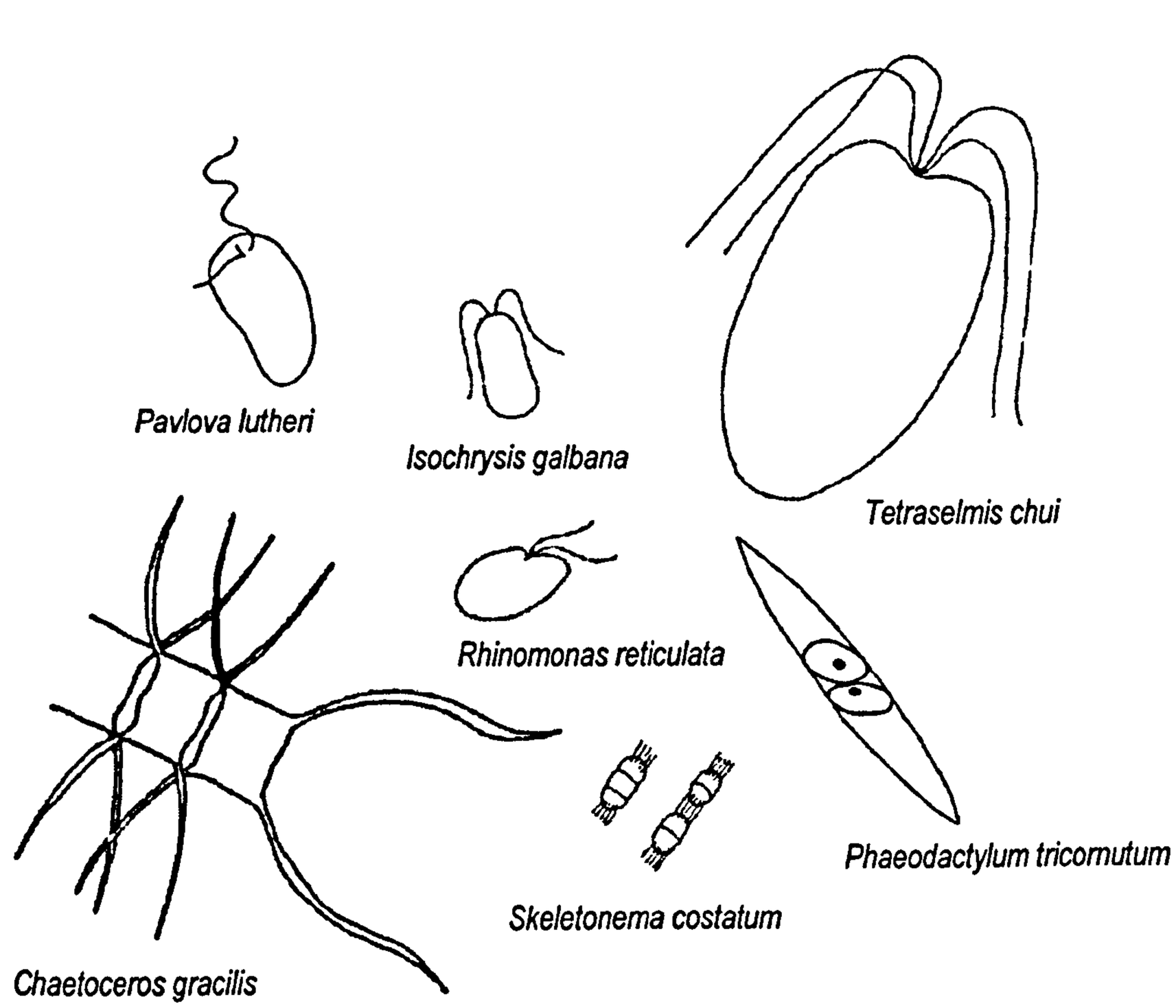


Figure II-3. Schematic representation of algal species used during experimentation of present work.

Algae cultures were always maintained at low density (i e, in early log phase) to ensure healthy cultures and reduce the probability of these to collapse. Large volume (20 dm^3) cultures were preferred since larger jars permit better aeration reducing cell accumulation and deposition rates. Achieving successful algal cultures requires maintenance of stable temperature and thorough innocuous incubation.

Although algal rearing facilities provided both at the Marine Research Centre in Galicia, Spain (CIMA-Corón) and in the School of Ocean Sciences in the UK (University of Wales, Bangor) were of the highest standards, algal quality was also assessed prior to all experiments. On several occasions, experiments had to be repeated or postponed in order to ensure the same culture conditions at comparable stages.

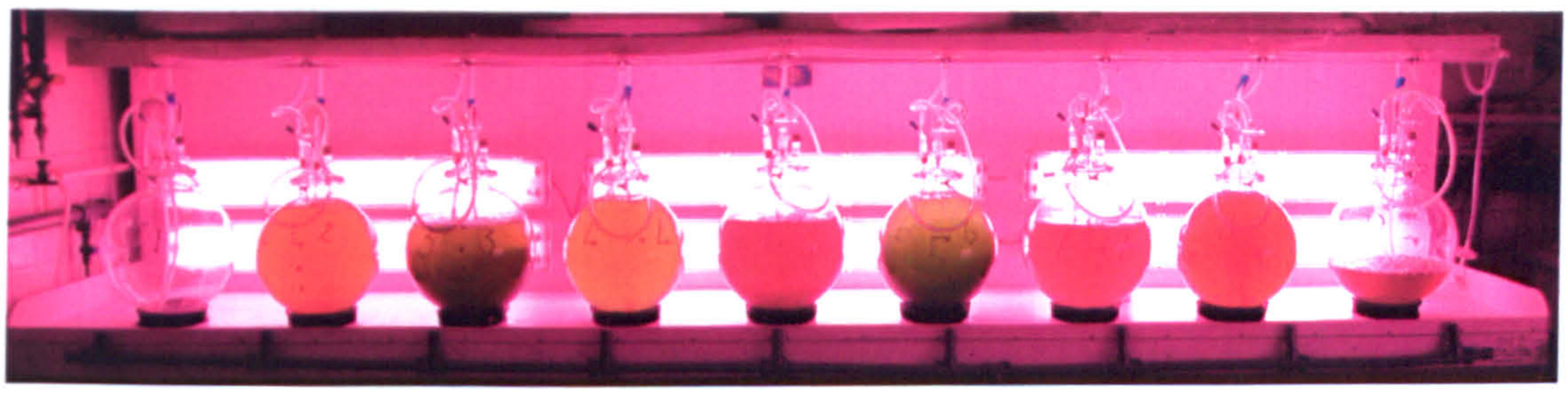
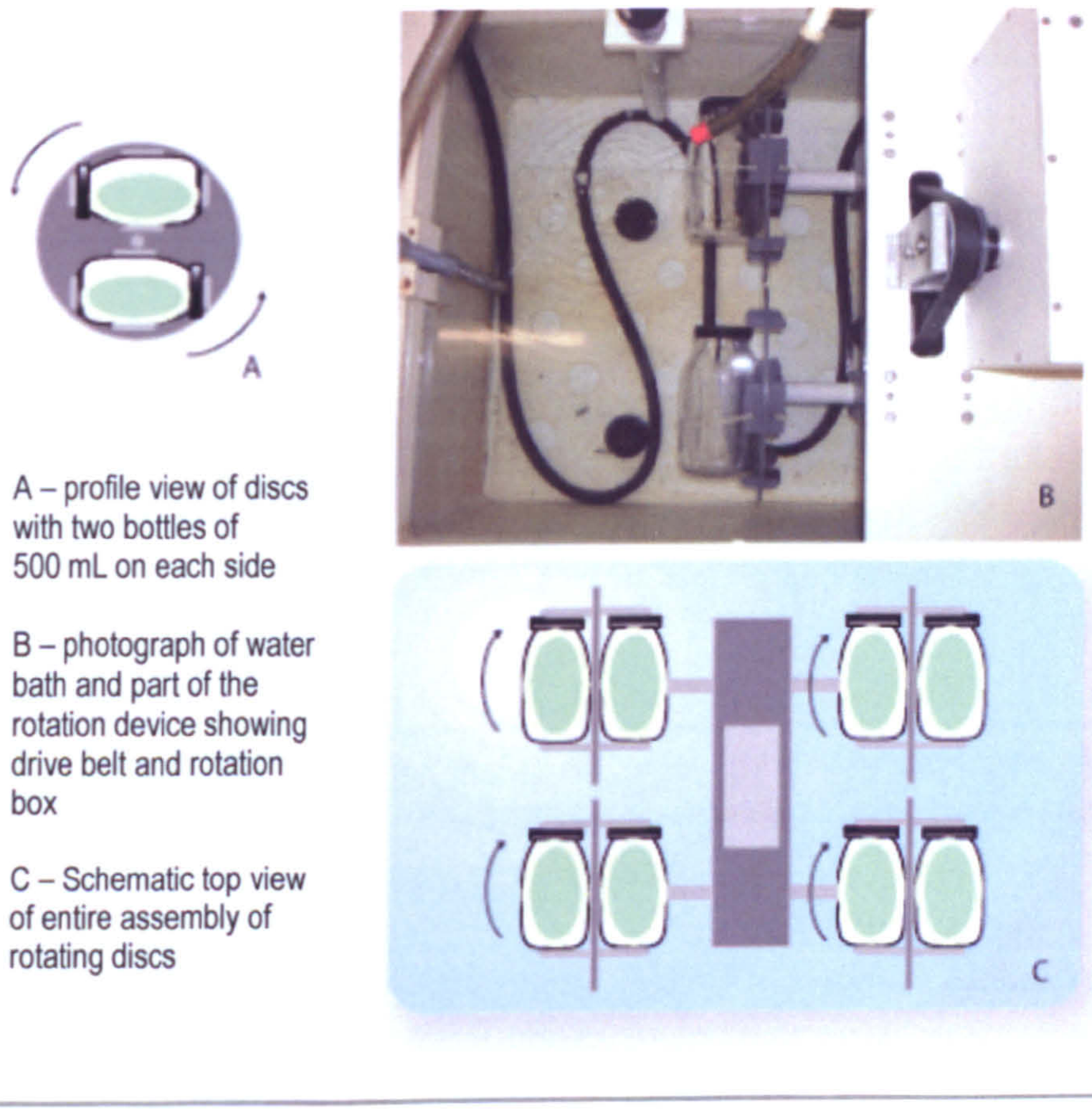


Figure II-4. Algal cultures of *Pavlova lutheri* (jars 2 and 4), *Tetraselmis chui* (jars 3 and 6), *Rhinomonas reticulata* (jars 5 and 7) and *Skeletonema costatum* (jars 8 and 9). (Phytoplankton laboratory in the School of Ocean Sciences, Univ. Wales – Bangor, photo kindly provided by Mr. Pedro Freitas)

II.4. ROTATION DEVICE FOR LABORATORY ZOOPLANKTON EXPERIMENTATION

Deposition of algal food in the bottom of the experimental vessels is one of the problems encountered when running zooplankton feeding experiments and may be addressed by using different methods and mathematical corrections (White 1976). In the current study, following the first experiments with *P. pollicipes* feeding, where a rotation wheel was not available, the need for a constant moving system in a controlled temperature environment was imperative. A system that could satisfy both conditions and still use the smallest space possible was designed.

A rotary system that worked inside a water bath was the best option and it is shown in Figure II-5. Comprising four rotating discs, two bottles of 500 mL could be attached to both sides of each disc, allowing experiments with 16 replicates. The elastic and attachments on each disc side were adjustable in order to carry a larger number of smaller vials and increase the number of replicates of smaller volumes. The motor was enclosed inside a water splash-proof box and its drive belts were designed in a way that the turn of the bottles would not exceed 1 rotation min^{-1}.



A – profile view of discs with two bottles of 500 mL on each side

B – photograph of water bath and part of the rotation device showing drive belt and rotation box

C – Schematic top view of entire assembly of rotating discs

Figure II-5. Rotation wheel device immersed in controlled temperature water bath for zooplankton experiments.

Aeration in the water bath was high to ensure constant and homogeneous temperature in the tank, while the motor was allowed to be on continuously throughout the entire experimental period, which in some cases exceeded the 17 days.

Light intensity in the tank was homogeneous, provided by fluorescent lamps attach to the ceiling of the laboratory. Despite the absent of a direct light source,

concentration of light upon a fixed spot on the flasks was possible, which would have attracted these phototropic larvae; yet by rotating the bottles, this device prevented the gathering of the larvae to a persistent place.

In order to maintain oxygen available to the larvae during experimentation, total volume of vials exceeded the larval/algal suspension final volume, i.e., some air was let inside the flasks, allowing aeration of cultures while turning in the immersed wheels.

II.5. LARVAL SIZE MEASUREMENTS

Fed and actively swimming larvae of both species were transferred to embryonic dishes with 3.9% ammonium formate isotonic solution. Although this is normally used for dry weight techniques, it also delays the loss of body fluids (Collis, 1991), which was important for size measurements particularly on soft body parts.

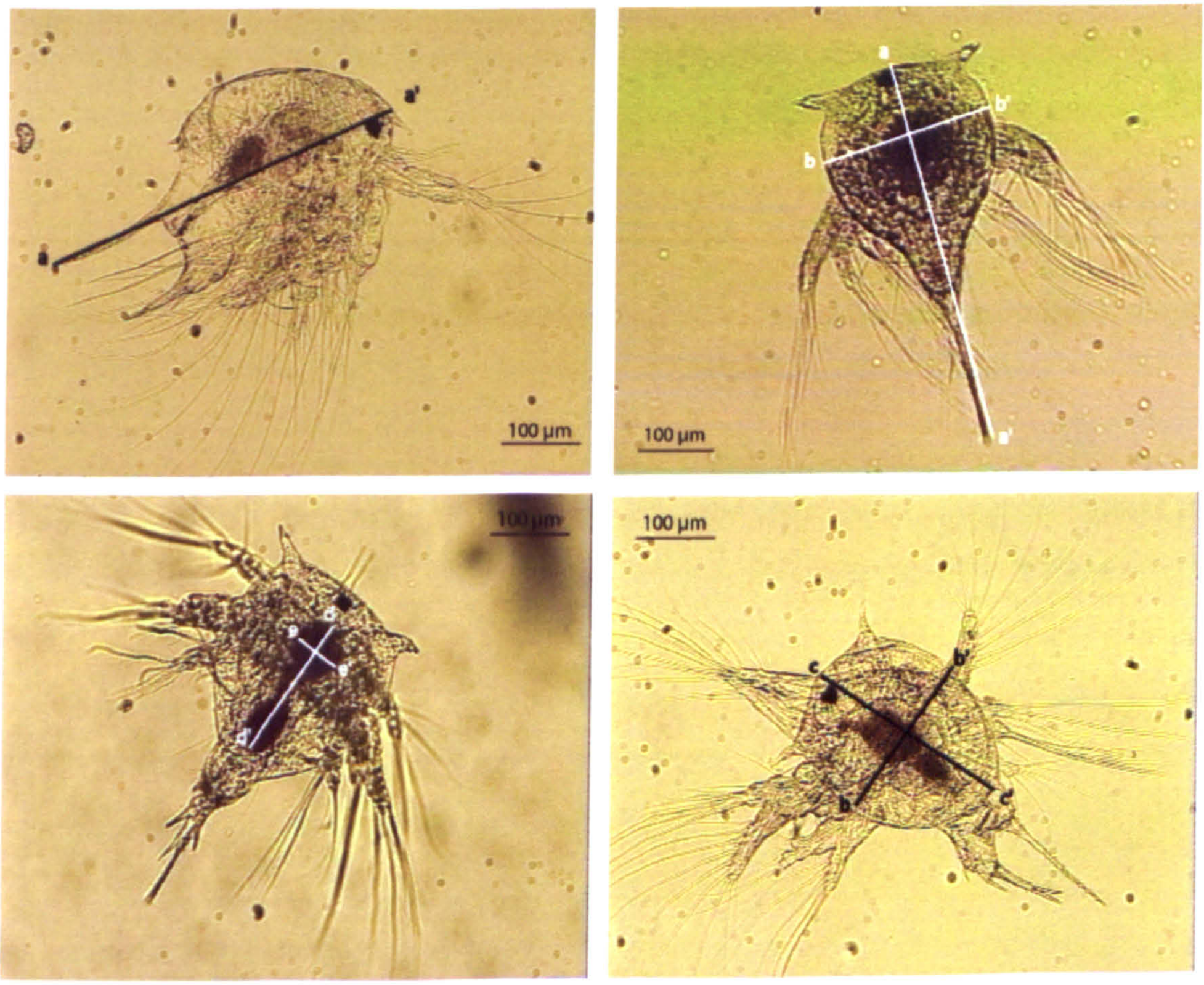


Figure II-6. *Elminius modestus* larvae: Measurements of (a-a') total lenght, (b-b') greatest carapace width, (c-c') metanauplii carapace shield length, (d-d') gut length, and (e-e') gut greatest width.

Overall body size of all stages were measured with two different eyepiece graticules in a Leica Laborlux S optical microscope. Both units were calibrated and presented different conversion factors for a 10-x objective (10.2 and 10, respectively).

The terms total length (L) and greatest width (W), as applied to barnacle nauplii, refer to the length of the longitudinal and dorso-ventral axes (Figure II-6). Carapace shield length (CSL) was assessed when it became defined, after stage NIII. Total gut length (GL) and width (GW) were also measured in larvae that had been feeding, the guts of which being apparently full.

III Feeding of *Pollicipes pollicipes* GMELIN 1790 (Cirripedia: Lepadomorpha) larvae

Feeding and development experiments were conducted with the Scalpellidae barnacle *Pollicipes pollicipes* larvae, using several laboratory reared microalgae. The flagellates *Rhinomonas reticulata* and *Tetraselmis chui* and the diatoms *Skeletonema costatum* and *Phaeodactylum tricornutum* were offered as monoculture food at initial densities of 100 cells μL^{-1}. Metanuaplii larvae did not feed on *P. triornutum* and feeding rates on *S. costatum* were highly variable. Due to large differences in cell size between the algal species, calculation of ingestion rates were based on cell volume which was more appropriate than cell density. Generally, the average feeding rates increased with stage and larvae were able to handle bigger cells, denoting a significant difference between NV and NII larvae and between NVI and all previous stages. Digestion times and assimilation efficiency were discussed using larval gut measurements.

The rate of development and survival of *P. pollicipes* larvae were measured for various algal diets. A constant rotating wheel device immersed in a temperature-controlled tank fulfilled experimental requirements, preventing decrease of algal food and promoting oxygenation of cultures. Mortality rate was highest during NI to NII moult. High proportions of *S. costatum* and *T. chui* retarded development. *R. reticulata* and a 5-algae mixture were the most successful diets, completing a larval life cycle within 14 days after hatching. Laboratory conditions of algal cultures may affect larval feeding and development rates. Links were found between the generalist diet of this cirripede species and their geographical distribution at locations of upwelling.

III.1. INTRODUCTION

The description of the feeding apparatus of barnacles has intrigued zoologists for almost a century (Lochhead 1936, Norris & Crisp 1953, Rainbow & Walker 1976, Yule 1982, 1984, Moyse 1987). Using scanning electron microscopy, Rainbow & Walker (1976) were able to resolve the topographical relationship of the limbs and other structures associated with feeding of barnacle nauplii, but discussion on the subject elsewhere was far from being exhausted. Description of the structures had to be combined with function analysis, i.e. observation of the animal's behaviour.

Barnacle larvae exhibit a jerky swimming movement, which results from a combined slightly out-of-phase rhythm of the mandibles, antennae and antennules. The

three thoracic limbs participate differently in the movement and while the antennae are mainly responsible for the larger propulsive forward stroke, the mandibles fold towards each other at the end of the anterior-posterior stroke, propelling water posteriorly, ventrally and anteriorly, resulting in a smaller propulsive movement. The antenulles produce a small arc on the propulsive stroke and, as they are mainly held forward, they serve to counterbalance and stabilise the movement of the ventral thoracic and caudal processes.

Reviewing several notable studies (Lockhead 1936, Norris & Crisp 1953, Gauld 1959, Rainbow & Walker 1976, Moyse 1984), Walker, Yule & Nott (1987) brought swimming and feeding behaviour together and concluded that the feeding mechanism of balanomorph larvae was driven essentially by the backward stroke of the antennae, which brings the particles within reach of the mandibles during the recovery stroke. Particles are then trapped towards the labrum during the enclosing process of the mandibles, limited by the mandibular endopods, at the end of the backward stroke. Labral setae and gland secretion and the continuous mandibular recovery stroke prevent particles being washed away during the powerful propulsive antennal stroke. Finally, the gnathobases, closer to the mouth region, force the food inside the animals mouth.

Yule (1982) presented a system to restrain individual larvae, which allowed direct observation and experimentation on a wide range of planktonic organisms, reducing damage to or disturbance of the animals, while applying various stimuli for microscopic, electrical or photographic recording. By detailed study of limb beat rate, swimming movement and capture of algae, he concluded that barnacle larvae are able to increase the swimming recovery stroke of the mandibles in order to control their feeding rate, depending upon the particle density in the suspension. Also, nauplii can reject particles, particularly from dense particulate suspensions, or discriminating food from non-food particles, thereby demonstrating selective feeding behaviour (Yule 1982).

Such observations have not been noted from scapellomorphic larvae, but lepadid nauplii show similar swimming strokes to that described from balanomorph larvae (Moyse 1987), which indicates a morphological consensus between thoracic planktotrophic barnacle larval swimming behaviour, this varying mainly in the beat rate ranges and swimming capabilities.

The size of food particles barnacle nauplii are able to ingest depends on the mouth size. Initially, Lochhead (1936) indicated that only cells or chains of diameter

smaller than 6 µm length up to 25 µm would fit the naupliar mouth of the *B. perforatus*. Moyse (1963) went further by testing seventeen different algal monocultures as food for four species of barnacles and concluded that diet preferences of barnacles reflected their geographical distribution and resulted from the size of the antennal intersetae space. Stone (1989) pointed out that the antennal filter mesh size not only differed between species but also within larval stages of the same species and therefore, diet preferences should be expected to change with larval development.

Ability to ingest a given planktonic cell does not necessarily mean that that cell is of dietary importance. Thus, experimentation on ingestion rates on different phytoplankton should be accompanied by tests of larval development success, as a better measurement of the nutritional value of the ingested material.

Molares (1994) used monocultures of the small flagellate *Isochrysis galbana* to grow *P. pollicipes* in mass culture, while Kugele & Yule (1996) used a mixture (1:2) of *Rhinomonas reticulata* and *Skeletonema costatum* when describing all larval stages of this species. Nevertheless, until the current study no research has been undertaken on *P. pollicipes* larval feeding habits. In view of future laboratorial work on this species, it is critical to determine successful conditions for obtaining higher and more consistent crops of viable larva.

Barnacle larval development is known to depend on numerous factors (Levin & Bridges, 1995), particularly temperature (Harms 1986, Venegas *et al.* 2000) and food availability (Lang *et al* 1980, Lang & Marcy 1982, Qiu 1997, Desai & Anil 2004) salinity (Walker & Lester 1998), and pollutants (Lam *et al.* 2000, Billingurst *et al.* 2001), just to mention the most studied. The present chapter focuses on the larval feeding during development of *P. pollicipes*, compares different algal diets under laboratory conditions, and discusses:

- the physical constraints of cell volume on ingestion rates,
- the nutritional constraints of algal diets on larval development rates.

III.2. METHODOLOGY

III-2.1 Preparation of larvae and feeding rates

Initial algal suspensions were diluted to prepare four different mono-algal suspensions, *Isochrysis galbana*, *Phaeodactylum tricornutum*, *Tetraselmis chui.*, and *Skeletonema costatum*, to a final concentration of 100 cells μL^{-1} each. This algal density has been indicated by several authors as optimal when rearing barnacle larvae *in vitru* (Kugele & Yule 1996).

For each algal regime, seven replicates were prepared, each containing 30 mL of food culture and 15 nauplii, each at the same larval stage, added to the cultures with a non-significant volume of seawater. Seven control bottles, each with 30 mL of culture, were prepared, without larvae, for each algal diet.

An initial 1000 µL sample was taken from each bottle, transferred to Eppendorf tubes and fixed with 100 µL of Lugol's iodine solution.

Bottles were left in a water bath, mixed with an air-stone, in a constant temperature room at 20°C. Bottles were regularly inverted and gently shaken to reduce algal deposition on the bottom of bottles and help maintain food concentrations.

After a period of 7 - 8 hours, 1000 µL sample from each algal suspension were placed in Eppendorf tubes and fixed with 100 µL of Lugol's iodine solution. The number of actively swimming nauplii in each bottle was also assessed and taken into consideration during the calculations. After gentle stirring of the Eppendorf tubes to homogenise samples, at least three drops were transferred to a modified Fuchs-Rosenthal haemocytometer and left to settle. Using a compound microscope, the algae present in a minimum of three medium squares (0.6 µL) were counted from each sample. Algal cell density (cells μL^{-1}) was then calculated allowing for the dilution by Lugol's iodine.

Clearance rates ($\mu L_{\text{volume swept clear}}$ nauplius^{-1} hour^{-1}) were calculated using the following equation:

$$F = \frac{V}{nT} \ln\left(\frac{E_0}{C_0} \times \frac{C_f}{E_f}\right) \qquad \text{...Equation III-1}$$

where V is experimental volume (μL), n number of nauplii, T experimental time interval (hours), E_0 and E_f initial and final concentrations (cells μL^{-1}) in the experimental vials and C_0 and C_f initial and final concentrations (cells μL^{-1}) in the control vials (White 1976, after Fuller & Clarke 1936, Lucas 1936, Fuller 1937)

The quantity of algae ingested (cells $nauplius^{-1}$ $hour^{-1}$) was estimated from the average concentration of available algae over the experimental period, as follows:

$$I = F \times \frac{(E_0 + E_f)}{2} \qquad \text{...Equation III-2}$$

III-2.2 Larval development and survival experiments

Since the main interest of this experiment was to test preference on food particles as structures, *I. galbana* used successfully by Molares (1994) on large cultures of *P. pollicipes*, was here substituted by another available small flagellate of similar dimensions, *R. reticulata reticulata* (R). Suspension (S) had the diatom *S. costatum* as a monoculture alternative. Kugele & Yule (1996) preferred a mixed suspension of *R. reticulata* and *S. costatum* (R+S) to rear *P. pollicipes* larvae. The diet proposed by Stone (1988) to cultivate *E. modestus* was also considered (R-T); higher development rates of these larvae were higher when offered a small flagelate (*Isochrysis sp.*) during the ealier naupliar stages and a larger one (*Rhodomonas sp.*) after larvae reach NIV.

Table III-3 summarises the 5 different diets used to grow *P. pollicipes* larvae. (R), (S) and (R or T) were monocultures of, respectively, the flagellate, *R. reticulata*, the diatom *S. costatum* and *R. reticulata* to the metanauplius stage followed by *Tetraselmis chuii* thereafter. (R+S) and (MIX) were mixed diets, respectively, *R. reticulata & S. costatum* (cell density ration of 1:2) and all available phytoplankton species in the laboratory (5 species in equal cell density proportions). The total cell density of each diet was always 100 cells μL^{-1}.

Each feeding regime was tested using three replicate cultures, each with 500 mL of algal suspension and, initially, 250 newly hatched *P. pollicipes* nauplii. The cultures were attached to a rotating wheel (describe above) and immersed in a

temperature controlled water bath at 17.5°C ± 0.5. A 12-hour light-dark cycle was followed throughout. Every two days, the cultures were poured through a 80 μm sieve and gently washed with fine-filtered (0.2 μm) and U-V irradiated seawater (UVFSW). The larvae were counted, staged and their mid-gut fullness observed under a dissecting microscope. Detritus, moults and dead nauplii were removed by pipette and the remaining larvae re-established in fresh culture media.

Table III-3. Abreviations of algal regimes used as food for *P. pollicipes* during larval development (see text).

Feeding regimes	Algal food (total density 100 cells·μL^{-1})		Reference
	Until larval stage III	After larval stage IV	
R	*R. reticulata**	*R. reticulata**	Molares 1994*
S	*S. costatum*	*S. costatum*	
R - T	*R. reticulata*	*T. chui***	Stone 1988**
R + S	*R. reticulata* + *S. costatum* 1:2	*R. reticulata* + *S. costatum* 1:2	Kugele & Yule 1996
MIX	*R. reticulata* + *S. costatum* + *T. chui* + *Chaetoceros neogracilis*+ *Pavlova lutheri* 1:1:1:1:1	*R. reticulata* + *S. costatum* + *T. chui* + *Chaetoceros neogracilis* + *Pavlova. lutheri* 1:1:1:1:1	

*Molares (1994) used a small flagellate of similar size *I. galbana*.
** Stone (1988) used the large flagellate of similar size *Rhodomonas sp*.

III.3. RESULTS

III-3.1 Larval measurements of *Pollicipes pollicipes*

Table III-4 andTable III-5 show the mean, the standard deviation and the range values (μm) of the total length (L), the greatest width (W), the cephalic shield length (CSL), the gut total length (GL) and the gut greatest width (GW) of *P. pollicipes*

nauplii (NII-NVI) (For methodology on larval measurements, please refer to Chapter II).

All measurements overlapped greatly between adjacent stages, which is particularly evident with stages NII and NIII showing the same maximal values. NVI showed the expected bending of the thoracic-ventral process, which made total length (L) sometimes difficult to measure in this stage. The flexure is preparative for the moult to cyprid when all the body eventually fits insides the folded carapace. Body sizes increased 4-17% with the greatest overall growth between stage NV to stage NVI.

Table III-4. Measurements of total length, greatest width and cephalic shield length of all feeding larval stages of *P. pollicipes*.

	total length (µm)				greatest width (µm)				carapace length (µm)			
stage	n	Mean	StDev	Range	N	Mean	StDev	Range	n	Mean	StDev	Range
Nii	34	379	30	285-459	34	218	21	143-255				
Niii	53	424	22	364-459	52	255	18	194-296				
Niv	25	442	25	408-479	25	289	25	235-337	27	289	26	235-326
Nv	54	472	31	347-530	57	338	30	255-398	56	330	24	275-398
Nvi	38	542	33	490-612	38	386	32	347-469	38	390	31	326-439

Table III-5. Measurements of total gut length and width of all feeding larval stages of *P. pollicipes*.

	gut total length (µm)				greatest width (µm)			
stage	n	Mean	StDev	Range	n	Mean	StDev	Range
Nii	18	97	33	61-153	18	57	16	36-82
Niii	22	103	19	61-133	22	80	21	61-122
Niv	21	167	50	102-255	21	81	32	31-143
Nv	22	216	52	102-286	22	112	20	82-153
Nvi	20	246	39	184-316	20	136	31	91-204

Kugele & Yule (1996) used L and W to indentify larval stages of *P. pollicipes*, by means of a linear discriminate function (LDF). In this study, Bonferroni pairwise comparison procedure (lack of fit test) was also significant at all levels ($F_{df=119[2,199]} = 2.59$, $p < 0.001$), indicating the multiregression function was appropriate to predict larval stages. The regression analysis was significant ($F_{df=2,199} = 796.72$, $p < 0.001$) and it is described as:

stage = - 3.06 + 0.00511 L + 0.0159 W

Respective coefficients are shown on Table III-6.

Table III-6. Multiple regression function between total length and greatest width (predictors) and naupliar stage (nauplii II-VI are 2-6, response) of *P. pollicipes*.

stage = -3.0649 + 0.0051 L + 0.0159 W

Predictor	Coef	SE Coef	T	p-value
Constant	-3.0649	0.2670	-11.48	<0.001
Length	0.005111	0.001099	4.65	<0.001
Carapace Width	0.015939	0.001068	14.92	<0.001

The gut volume volume was calculated assuming the gut shape approximate to an ellipsoid (slightly strangulated in the metanauplii), there is an apparent exponential increase, but variability within each stage was extremely high, especially on stage NIV (Table III-7). Results of gut volume were logarithmically transformed (Natural ln μm^3) and were significantly correlated with larval stage (Spearman rank correlation = 0.994, $p < 0.001$) Residuals of the regression analysis were approximate to a normal distribution ($A^2 = 0.715$, $p = 0.060$) and the regression function is expressed by the following equation:

$$\text{Gut volume} = e^{10.5+0.692 stage}$$

Table III-7. The means, standard deviation and range of larval gut volume (μm^3) of actively swimming *P. pollicipes* larvae considering the total length and greatest width of an ellipsoidal shaped gut.

larval stage	Gut volume ($x10^5 \mu m^3$)			
	n	Mean	StDev	Range
NII	18	1.8	1.1	0.4 – 3.6
NIII	22	3.8	2.2	1.2 – 8.8
NIV	21	8.0	8.1	5.5 – 27.2
NV	22	15.2	8.2	3.6 – 35.0
NVI	20	26.1	13.3	8.1 – 53.3

III-3.2 Feeding rates of *Pollicipes pollicipes* larvae on algal monocultures

Figure III-7 summarises the clearance rates all larval stages of *P. pollicipes* when fed *I. galbana*, *P. tricornutum*, *S. costatum* and *T. chui* at a nominal algal cell density of 100 cells μL^{-1}. Larvae older than stage III never consumed *P. tricornutum* therefore these results were excluded from the analysis.

P. pollicipes clearance rates on *I. galbana* and on *T. chui* increased significantly with stage (Spearman Rank correlation = 0.814, $p < 0.001$ and 0.414, $p = 0.013$, respectively). Although the mean larval clearance rates on *S. costatum* suggest an increase with larval stage, such was not significant (Spearman rank correlation = 0.269, $p = 0.124$). *S. costatum* tends to form chains, sometimes very long, which are difficult to count and this has undoubtedly resulted in the high variability exceeding that of any trend in clearance rate.

Clearance rates reflect cell dimensions, particularly for the metanauplii (NIV – NVI), i.e. the smallest cells, *I. galbana*, elicited clearance rates between 2.4 and 6.3 times greater than did the largest cells, *T. chui*. The small-celled but chain-forming *S. costatum* ellicited intermediate clearance rates except at stages II & III.

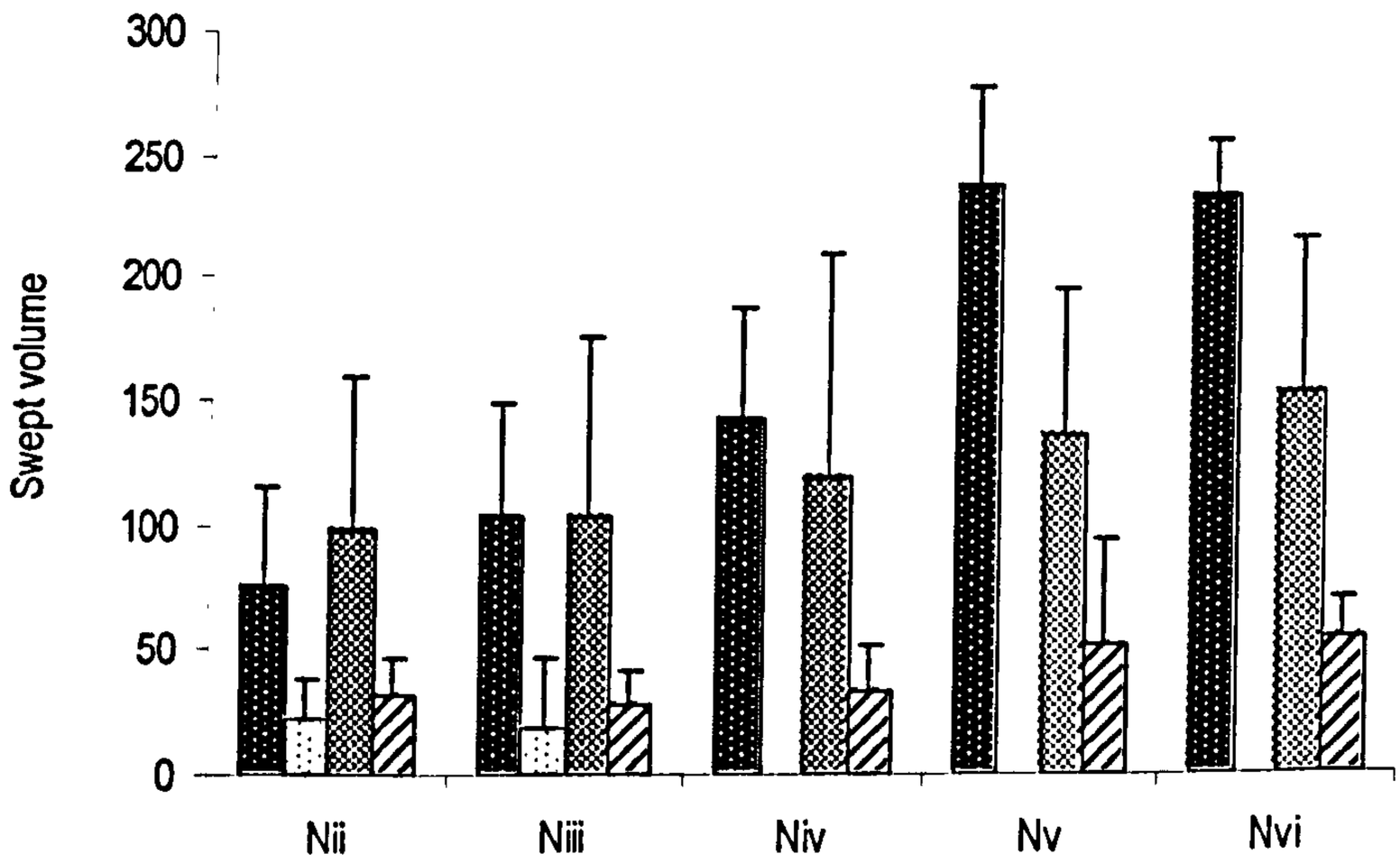


Figure III-7. Mean (bars = standard deviation) clearance rates ($\mu L_{volume\ swept\ free}$ animal^{-1} hr^{-1}) of *P. pollicipes* larval stages feeding on *I. galbana* (white dots), *P. tricornutum* (confetti), *S. costatum* (checker), and *T. chui* (upward lines). *P. tricornutum* was only consumed at nauplii II and III.

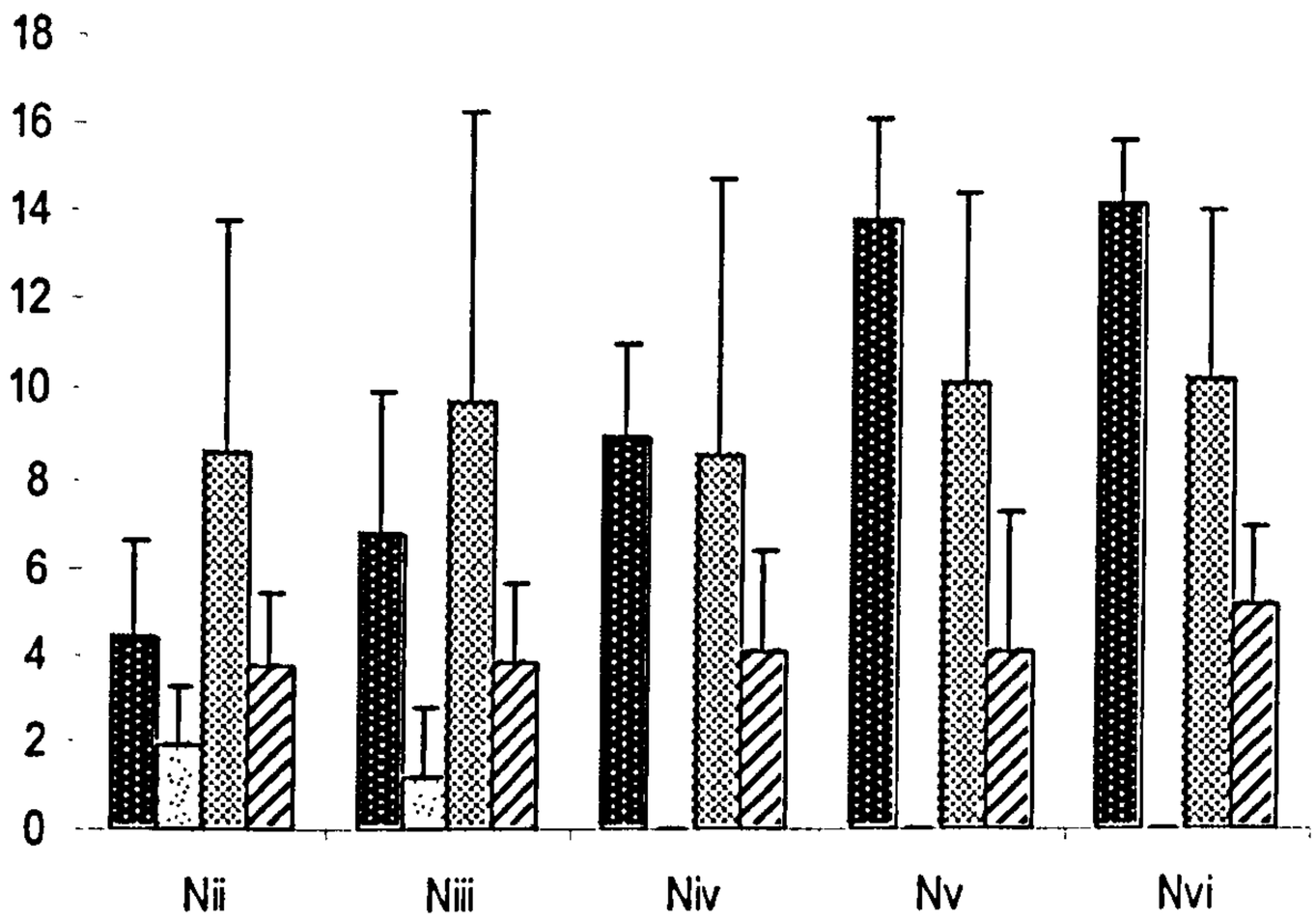


Figure III-8. Mean(bar = standard error) ingestion rates (x10^3 cells animal^{-1} hour^{-1}) of *P. pollicipes* larval stages feeding on *I. galbana* (white dots), *P. tricornutum* (confetti), *S. costatum* (checker), and *T. chui* (upward lines). *P. tricornutum* was only consumed at nauplii II and III.

The cell ingestion rates (Figure III-8) largely replicate the pattern shown for clearance rates in Figure III-7 because all initial cell densities were adjusted to 100 cells μL^{-1}. The increase in ingestion rate for larvae feeding on *I. galbana* was very clear but less obvious for larvae feeding on *T. chui*, given the scale, while ingestion rates for larvae feeding on *S. costatum* varied little with stage at 8-10 x10^3cells animal hour^{-1}.

The mean values and standard deviations of *P. pollicipes* clearance rate across the algal diets are shown on Table III-8(a). Residuals from the means were largely normally distributed but Bartlett's test suggested significant heterogeneity of variance ($\chi^2 = 22.590$ $p < 0.001$). Variability of clearance rates on *S. costatum* was particularly high (cv% from 41 – 75%) and that for *I. galbana* was also quite variable between stages (cv% from 10 – 53%). Table III-8 (b) shows a Factorial (algal diet x naupliar stage) analysis of variance of mean clearance rates (μL animal^{-1} hour^{-1}) for *P. pollicipes* larvae. Despite the wide differences in variance, the interaction term proved highly significant indicating the need for further analysis of the simple effects means. The nature of the interaction is discernible in Figure III-7 and Table III-8 (a) where the clearance rate for larvae feeding on *S. costatum* exceeds that for larvae feeding on *I. galbana* for NII & III but the opposite is true for the metanauplii (NIV – NVI).

Table III-8. Clearance rates (µL animal^{-1} hour^{-1}) of *P. pollicipes* feeding on *I. galbana*, .*S. costatum*, and *T. chui* monocultures.

(a). Mean and standard deviation (in brackets)

	Pollicipes pollicipes larval stages				
	NII	NIII	NIV	NV	NVI
I. galbana	75.4 (40.03)	103.2 (44.88)	142.3 (44.99)	233.1 (23.21)	158.2 (76.78)
S. costatum	97.9 (62.05)	121.2 (59.59)	120.0 (90.34)	135.1 (60.39)	152.5 (63.33)
T. chui	32.0 (14.58)	28.4 (13.26)	33.0 (18.83)	36.9 (17.74)	54.5 (16.23)

(b) Factorial analysis of variance

Source	DF	Sum of Squares	Mean Square	F	p
Stage	4	96006	23554	11.01	<0.001
Alga	2	274250	137464	64.28	<0.001
Interaction	8	73013	9127	4.27	<0.001
Error	89	190337	2139		
Total	103	633606			

Table III-9 presents all pairwise comparisons of the simple effects clearance rate means using Tukey's method. Clearance rates for NVI larvae feeding on *I. galbana* were significantly higher than those for all other stages and diets except for NV larvae feeding on *I. galbana*. Except at NII, larvae feeding on *T. chui* exhibited significantly lower clearance rates than on any other algae. The significant increase in clearance rate on *T. chui* with larval stage has been obscured by the higher average variance used in the ANOVA (see above, Table III-8 (b)). Differences amongst stages NII and NIII were only significant for higher clearance rates on *S. costatum* comparatively with those on *T. chui*.

Table III-9. Pairwise comparisons (using Tukey's method) between clearance rate means (µL animal^{-1} hour^{-1}) for *P. pollicipes* larvae (nauplii II – VI) feeding on *I. galbana* (Iso), *S. costatum* (Ske), and *T. chui* (Tet). Column means (see Table III-2(a)) have been subtracted from row means. A difference between means of 86.17 µl hr^{-1} is required for significance at the 5% level. Figures in bold denote significant differences.

	NII Iso	NII Ske	NII Tet	NIII Iso	NIII Ske	NIII Tet	NIV Iso	NIV Ske	NIV Tet	NV Iso	NV Ske	NV Tet	NVI Iso	NVI Ske
NII Ske	22.50	–	–	–	–	–	–	–	–	–	–	–	–	–
NII Tet	- 43.37	-65.88	–	–	–	–	–	–	–	–	–	–	–	–
NIII Iso	27.81	5.30	71.18	–	–	–	–	–	–	–	–	–	–	–
NIII Ske	45.83	23.33	**89.20***	18.02	–	–	–	–	–	–	–	–	–	–
NIII Tet	-46.95	-69.46	-3.58	-74.76	**-92.78***	–	–	–	–	–	–	–	–	–
NIV Iso	66.88	44.37	**110.25***	39.07	21.05	**113.83***	–	–	–	–	–	–	–	–
NIV Ske	44.64	22.14	**88.02***	16.83	-1.19	**91.59***	-22.2	–	–	–	–	–	–	–
NIV Tet	-42.42	-64.93	0.95	-70.23	-88.25	4.53	**-109.3***	**-87.06***	–	–	–	–	–	–
NV Iso	**161.74***	**139.23***	**205.11***	**133.93***	**115.91***	**208.69***	**94.9***	**117.10***	**204.16***	–	–	–	–	–
NV Ske	59.70	37.20	**103.08***	31.89	13.87	**106.66***	-7.2	15.06	**102.13***	**-102.0***	–	–	–	–
NV Tet	-38.54	-61.04	4.83	-66.35	-84.37	8.41	**-105.4***	-83.18	3.88	**-200.3***	**-98.24***	–	–	–
NVI Iso	**157.68***	**135.17***	**201.05***	**129.87***	**111.85***	**204.63***	**90.8***	**113.04***	**200.10***	-4.1	**97.98***	**196.22***	–	–
NVI Ske	77.12	54.62	**120.49***	49.31	31.29	**124.07***	10.2	32.48	**119.54***	-84.6	17.42	**115.66***	-80.6	–
NVI Tet	-20.92	-43.42	22.46	-48.72	-66.74	26.04	**-87.8***	-65.55	21.51	**-182.7***	-80.62	17.63	**-178.6***	**-98.03**

Algal monocultures were given to the larvae at the same cell density (100 cells μL^{-1}). However, the large difference in cell size between the species means that the algal volume density on offer to the larvae (a better indication of nutrient availability than cell density) differed greatly between species. From cell measurements and the closest geometric shape, *I. gabana* (ellipsoidal), *S. costatum* (cylindrical) and *T. chui* (ellipsoidal) have cell volumes of 21, 64 and 500 μm^3, respectively.

The change in cell volume ingestion rates for *P. pollicipes* larvae fed on the 3 algal species is shown on Figure III-9. Despite the lower clearance rates for larvae fed on *T. chui* (see Table III-8(a)), the large size of *T. chui* confers by far the highest volume ingestion rates across all larval stages. The smallest volume ingestion rate comes from feeding on the tiny *I. galbana*. Figure III-9 also indicates that volume ingestion increased with larval stage. The increase was significant for *I. galbana* (Spearman ranked correlation = 0.846, $p < 0.001$) but was not significant for larvae fed either *S. costatum* or *T. chui* (Spearman ranked correlation = 0.210, $p = 0.226$ and 0.041, $p = 0.818$, respectively). The variability in cell volume ingestion rates for larvae fed *S. costatum* and *T. chui* was much higher than for those fed *I. galbana*, thus obscuring any steadily increasing trend.

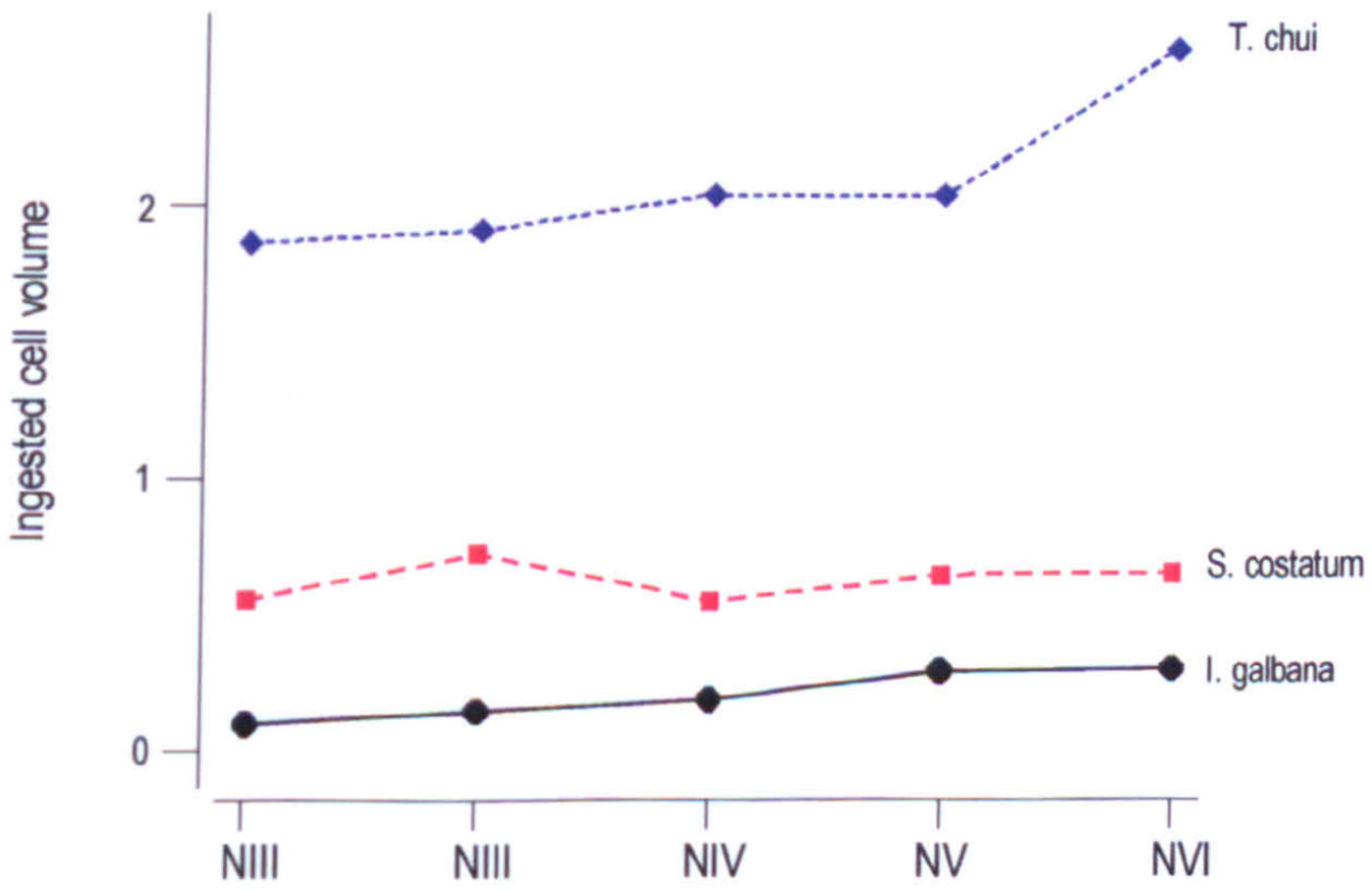


Figure III-9. Mean Ingested cell volume ($x10^6$ μm^3 $animal^{-1}$ $hour^{-1}$) by *P. pollicipes* larvae feeding on monocultures of three different algal species at 100 cells μL^{-1}: *I. galbana* (circles), *S. costatum* (squares), and *T. chui* (diamonds).

Table III-10 (a) shows the average volume ingestion rates for all naupliar stages. The standard deviations generally increased with magnitude of the mean with ingestion rates on *S. costatum* and *T. chui* exhibiting equivalent ranges of relative variability (cv% from 38 – 73% and from 33 – 76% respectively) with that for larvae feeding on *I. galbana* generally lower (cv% from 10 – 56%).

A Log_{10} transformation was successfully used to stabilise the variance (Levene's statistic for equal variance = 1.28, $p = 0.236$) prior to factorial analysis. Table III-10(b) shows the resulting factorial ANOVA indicating a lack of significant interaction ($F_{8,89} = 1.69$, $p = 0.111$). Both main effects were significant (Table III-10(b)) and multiple comparisons of the main effects were undertaken in the Log_{10} scale following Tukey's procedure. On average, *P. pollicipes* larvae ingested greater cell volumes as they became bigger. However, the rates only differed significantly (within the variability of the data) for NVI compared to all other stages but NV and for NV compared to NII (Table III-10(c)).

The clear differences (see Figure III-9 and Table III-10(a)) in larval ingestion of the 3 algal species were confirmed by the main effect comparisons (Table III-4(c)) with significant differences evident between all 3 species of algae. As expected, cell volume ingested by *P. pollicipes* larvae followed individual algal cell volumes ($r = 0.714$, $p < 0.001$ for $df = 102$).

Table III-10. Cell volume ingestion rate for *P. pollicipes* larval stages feeding on three different algal monocultures (*I. galbana*, *S. costatum*, and *T. chui*) at a cell density of 100 cells μL^{-1}

(a) Means ($x10^6$ μm^3 $animal^{-1}$ h^{-1}) and standard deviations (in brackets)

	Pollicipes pollicipes larval stages				
	NII	NIII	NIV	NV	NVI
I. galbana	0.09 (0.05)	0.14 (0.07)	0.19 (0.04)	0.29 (0.05)	0.30 (0.03)
S. costatum	0.52 (0.31)	0.69 (0.32)	0.51 (0.37)	0.61 (0.26)	0.61 (0.23)
T. chui	1.12 (0.51)	1.15 (0.54)	1.23 (0.69)	1.23 (0.94)	1.55 (0.52)

(b) Factorial (algal diet x naupliar stage) analysis of variance table after Log_{10} transformation.

Source	DF	SS	MS	F	p
Stage	4	1.11519	0.27654	3.99	0.005
Alga	2	10.88397	5.44667	78.58	<0.001
Interaction	8	0.93888	0.11736	1.69	0.111
Error	89	6.16871	0.06931		
Total	103	19.10674			

(c) Main effects means and their pairwise comparisons (by Tukey's method). Figures represent means or differences (column – row means) in the Log_{10} scale.

	STAGE				
	NII	**NIII**	**NIV**	**NV**	**NVI**
Mean	5.496	5.619	5.614	5.729	5.794

	NII	NIII	NIV	NV
NIII	0.132	-	-	-
NIV	0.118	-0.014	-	-
NV	0.233	0.102	0.115	-
NVI	0.299	0.167	0.181	0.066

difference required for significance at 5% level = 0.135

	ALGAL DIET		
	I. galbana	*S. costatum*	*T.chui*
Mean	5.239	5.688	6.026

	I. galbana	*S.costatum*
S. costatum	0.451	-
T. chui	0.786	0.335

difference required for significance at 5% level = 0.151

III-3.3 Development and survival rates of *Pollicipes pollicipes* larvae under different algal food regimes

Within three days of hatching, NI larvae were not found in any of the cultures. Since no dead NI larvae were recovered from the cultures it can be assumed that the larvae were in good condition at release and successfully developed a functional gut during the moult to NII. Table III-11 shows that all living nauplii had metamorphosed from NII between day 6 and 9 although by far the majority had done so by day 3. Mortality over the first 3 days was over 30% but only 6.5% over the next 3 days. Thereafter, survivorship decreased steadily until the end of the experiment when only 14.4% of the initial 3750 larvae had survived.

Table III-11. Average percentage survival of *P. pollicipes* larvae through the 14 day experimental period .

Days	NII	NIII	NIV	NV	NVI	Total
3	7.8	60.4				68.2
6	1.2	24.7	35.5	0.3		61.7
9		5.2	15.9	20.6	2.1	43.8
12		1.4	11.6	9.2	4.2	26.4
14				2.5	11.9	14.4

The number of surviving larvae in each replicate culture on each day is shown in Figure III-10. Three types of response are suggested and the relationships they represent have been drawn (by eye) on Fig III-12. The first type, larvae fed on monocultures of *R. reticulata* and those fed on the mixture of 5 algae, is consistent with a constant mortality rate leading to a linear decrease in the numbers of larvae surviving with time. Larvae initially fed *R. reticulata* and then switched to *T. chui* at the metanauplius stage show a linear decrease in numbers over the first 9 days but then a much more rapid decline in numbers to leave few survivors by day 14. Those larvae fed either *S. costatum* or a mixture of *R. reticulata* and *S. costatum* show little, if any, mortality from day 3 to 6 but thereafter show a rapid, linear decline in surviving numbers with time, also ending up with very small numbers of survivors by the fourteenth day.

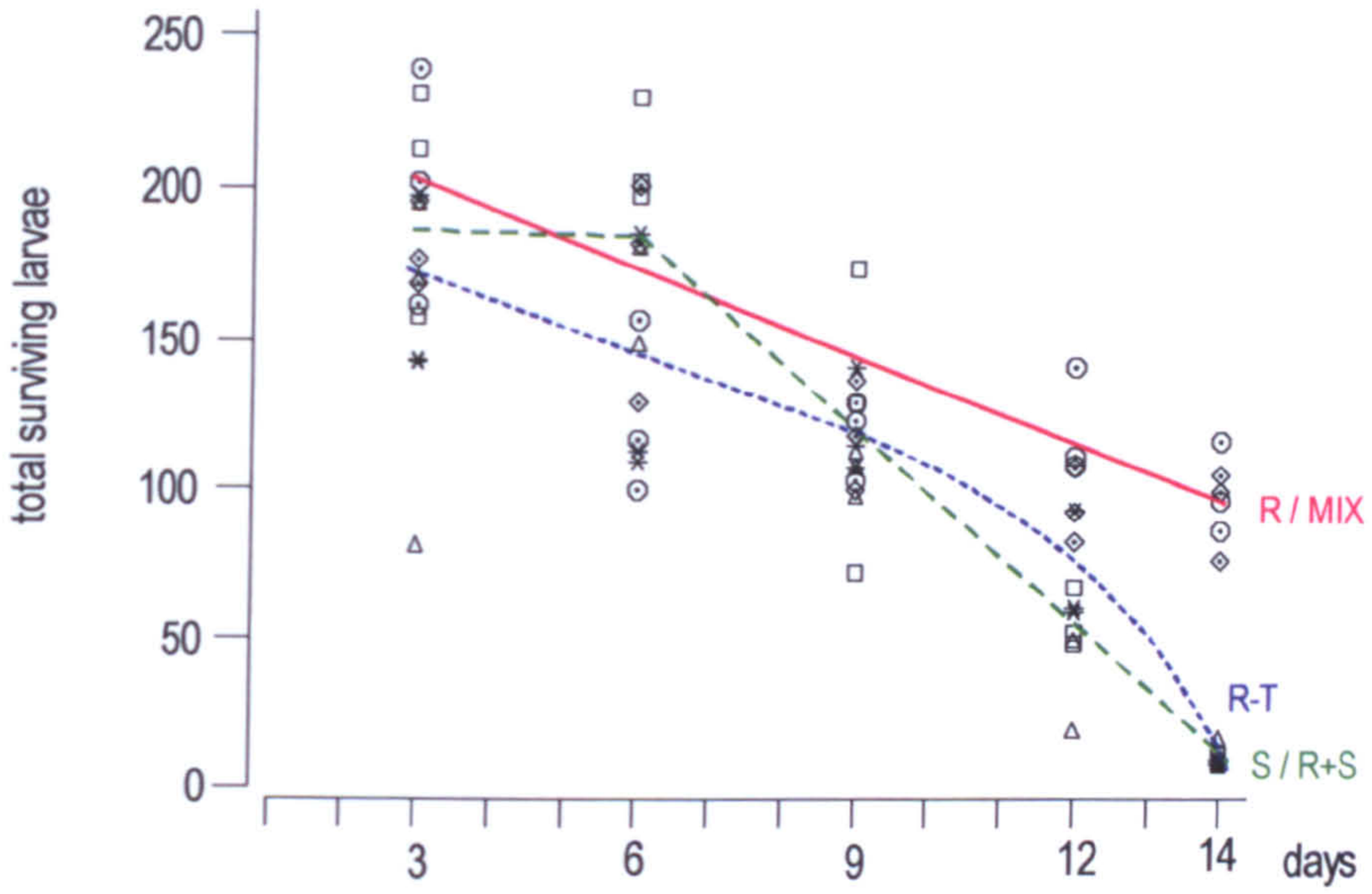


Figure III-10. Survival of *P. pollicipes* larvae on 5 different diets. R = *R. reticulata* (dot circles), MIX = mixture of 5 algae (dot diamonds), R-T = *R. reticulata* followed by *T. chui* (asterisks), S = *S. costatum* (triangles), and R+S = mixture of *R. reticulata* and *S. costatum* (squares). Lines fitted by eye to show general trends.

To analyse differences between diets for comparable regions of the survivorship curves (Figure III-10) regression analysis was used. All the observations for *R. reticulata* monocultures and the mixed algal diet were used, as were days 6 – 14 for the *S. costatum* monoculture and the *R. reticulata* and *S. costatum* mix but only days 3 – 9 for the *R. reticulata* to *T. chui* switch diet. For each diet, residuals from the regressions of numbers surviving against time showed no systematic departure from a linear trend and were normally distributed around their regression fits (Anderson-Darling $A^2 = 0.366$, $p = 0.423$, $n = 63$). Furthermore, the residual variances from the regression fits for each diet were approximately equal (Bartlett's $\chi^2 = 6.34$, $p = 0.175$). Regression fits for each diet were thus compared by two factor analysis of variance with time from hatch as the co-variate. Table III-12(a) details the fitted slopes and intercepts. Slopes ranged from -6.78 nauplii d^{-1} for the first few days on the *R. reticulata* to *T. chui* switch diet to -24.93 nauplii d^{-1} for the *R. reticulata* plus *S. costatum* diet (equivalent to mortality rates of 2.7% to 10% d^{-1}). Intercepts generally underestimated the initial number (250) of larvae except for the *S. costatum* monoculture and the *S. costatum* & *R. reticulata* mixture diets.

Table III-12. Regression analysis for *P. pollicipes* naupliar survivorship with five different diets

(a) Regression slopes and intercepts.

Diet	Slopes (+SE)	Intercepts (+SE)
Mixture	-9.10 ± 1.61	203.41 ± 15.27
R. reticulata	-7.50 ± 1.61	190.63 ± 15.27
R -> T	-6.78 ± 3.36	172.11 ± 19.33
R + S	-24.93 ± 2.35	347.58 ± 21.64
S. costatum	-20.71 ± 2.35	283.57 ± 21.64

(b) Factorial analysis of variance table

Source	DF	Seq SS	Adj SS	Adj MS	F	p-value
Diet	4	31865	25436	6359	10.42	<0.001
Day	1	116279	105585	105585	173.02	<0.001
Interaction	4	34907	34907	8727	14.30	<0.001
Error	53	32344	32344	610		
Total	62	215395				

(c) Comparison of intercepts to the average intercept of 239.46 ± 9.43 nauplii.

Diet	Difference in Intercept	SE of Difference	t	p-value
Mixture	-36.05	15.27	-2.36	0.022
R. reticulata	-48.83	15.27	-3.20	0.002
R - T	-67.35	19.33	-3.48	0.001
R + S	108.12	21.64	5.00	<0.001
S. costatum	44.11	21.64	2.04	0.046

(d) Comparison of slopes to the average slope of -13.80 + 1.05 nauplii day^{-1}.

Diet	Difference in Slope	SE of Difference	t	p-value
Mixture	4.71	1.63	2.89	0.006
R. reticulata	6.31	1.63	3.88	0.000
R - T	7.03	2.81	2.50	0.015
R + S	-11.13	2.10	-5.29	0.001
S. costatum	-6.91	2.10	-3.29	0.002

Table III-12(b) shows a significant interaction term ($F_{df=4,53} = 14.3$, $p < 0.001$) between factors diet and day, indicating that at least one slope differed significantly from at least one other. As anticipated, the overall regression was significant ($F_{df=1,53} = 173.02$, $p < 0.001$). Individual intercepts and slopes regarding each diet were then compared to their respective survival averages in Table III-12(c) & (d).

All intercepts, which estimate the initial numbers of larvae in cultures, were negative, showing a linear decrease in numbers from the start of the experiment. The intercepts for *R. reticulata* monocultures, the mixed algal diet and the early days of the *R. reticulata* to *T. chui* switch diets were significantly lower than the average but differed from each other by, at most, 31 nauplii (See Table III-12(c)). Such lower than average intercepts are indicative of a high rate of mortality of NI & NII nauplii over the first 3 days which were not sampled during the experiment.

The larvae grown on monocultures of *S. costatum* and the *S. costatum* and *R. reticulata* mixture exhibited significantly higher than average intercepts. The intercept for larvae on the *S. costatum* monoculture diet is considerably lower (64 nauplii less, see Table III-12(c)) than that for larvae on the mixed *S. costatum* and *R reticulata* diet. The higher than average intercepts are indicative of the better than average survival of the nauplii on those diets over the first six days. Apart from the initial mortality shown for all diets, systematic mortality was not evident in cultures fed these two diets until day 9.

When considering slopes comparisons, the same two groupings of response between diets were apparent. All slopes differ significantly from the average slope (see Table III-12(d)). Those for larvae fed the *R. reticulata* monoculture, the mixed culture and the *R. reticulata* to *T. chui* switch diets are all significantly greater than the average and do not differ significantly from each other. Larvae fed the other two diets showed significantly lower than average survivorship slopes, again not differing significantly from each other. When fed a mixture of algal species or a monoculture of *R. reticulata* mortality rates across all naupliar stages did not differ significantly, averaging 7.8 nauplii d^{-1} (3.1% d^{-1}, see Table III-12(a)). When the diet consisted of at least 66% *S. costatum* (by cell density) then mortality at stage NII – NIII was negligible but metanaupliar mortality (NIV – NVI) was much higher than the other three diets, averaging 22.8 nauplii d^{-1} (9.1% d^{-1}, see Table III-12(a)).

Table III-13 shows the peak occurrence of the various naupliar stages fed the 5 different diets. The *R. reticulata* monoculture and the 5-species mixture diets produced very consistent development over the 14-days experimental period. Indeed, substantial numbers of NVI nauplii were only ever developed on these two diets and only the *R. reticulata* monoculture produced any cypris stages. Cultures involving high proportions of *S. costatum* were retarded in development after the earlier naupliar stages and virtually no development was demonstrable for cultures where *R. reticulata* was replaced with *T. chui*.

Table III-13. Day from release when *P. pollicipes* naupliar stages showed peak abundances when fed five different algal diets. R = *R. reticulata*, S = *S. costatum* and T = *T. chui*.

Diet	NII	NIII	NIV	NV	NVI
R. reticulata	1	3	6	9	14
S. costatum	1	3	9	14	14
R -> T	1	3	6	9	-
R + S	1	3	6	14	14
Mixture	1	3	6	9	14

Figure III-11 illustrate relative abundances (per day) of the larval stages of *P. pollicipes* under the different feeding regimes. Until 9 days after release, development rate was slower on diets with *S. costatum*, although at that time mortality was not very different amongst all tested cultures. As expected, both regimes with *R. reticulata* show the same pattern in larval abundances, with very low if any mortality in moult from stage NIV to NV. After day 9, NV do not survive on *T. chui* nor on *S. costatum* (mono or mixed with *R. reticulata*), and very few larvae are able to moult. The diets that lead to the slower development rate were those with *S. costatum*, by showing a higher proportion of NIV on day 9, instead of NV. Diets *R. reticulata* and mixed algae were the most successful for rearing *P. pollicipes* larvae, completing the larval cycle at 17.5°C within 14 days after hatch.

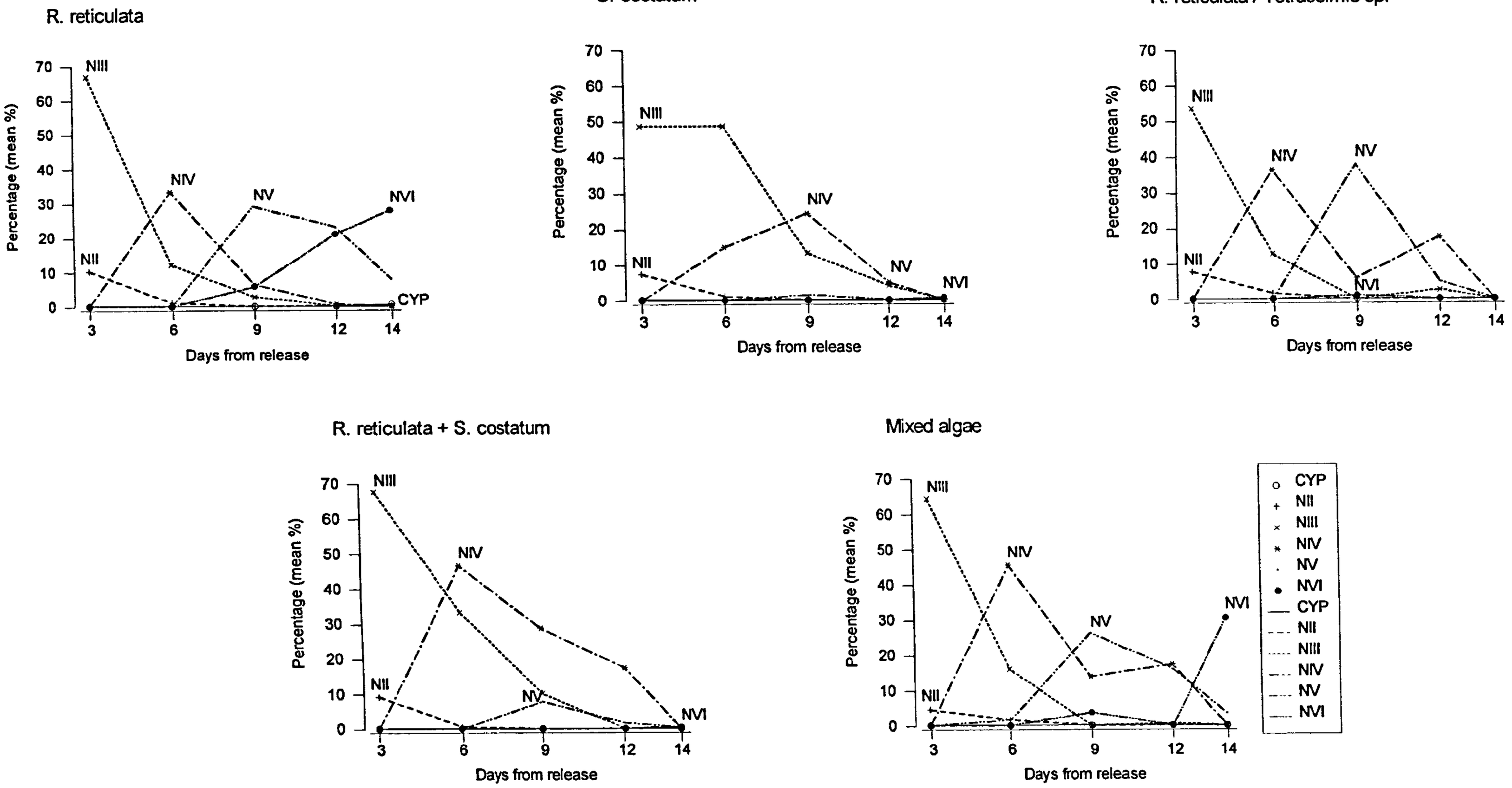


Figure III-11. Relative abundances (%) of *P. pollicipes* nauplii under five different food regimes during 14 days from larval release. Mean percentages values refer to initial total number of nauplii, 250 per replicate

III.4. DISCUSSION

Size of barnacle larvae is a result of temperature, light intensity and food concentration (Knight-Jones & Waugh 1949, Sandison 1967, Moyse 1963, Lewis 1975b). Although the range of total length (L) and maximum width (W) was greater in the present study, the mean values were approximately comparable to those presented by Molares (1994) and Kugele & Yule (1996). Larvae were staged based on morphological structure (as opposed to being adjusted to a stage predicted by a linear discriminant function), therefore the measurements derived in this study may reflect more accurately the variability of the population reared in laboratory. As suggested by Kugele & Yule (1996), larvae obtained from tank maintained adults were 10% smaller than those produced from adults in their natural environment, from which we can infer that larvae obtained from extracted egg sacs would show the same discrepancy if not higher. In absolute terms, the same range of sizes would be expected in the water column, although with a greater proportion of average-size individuals, particularly in the later stages where selection pressure has exerted more influence during the life-cycle.

Taking into consideration equal initial cell densities, the different cell ingestion rates are problematic. Analysis of control data, i.e. variation in the algal cultures without larvae, shows that *T. chui* cultures thrived, almost doubling in testes with NII larvae, *S. costatum* cultures grew on NII and NIII experiments and *I. galbana* density reduced in all experimental control vessels (Figure III-12). These systematic differences derived from the original algal cultures used, which were at different logarithmic growth phases, i.e. *I. galbana* was a comparatively older culture than *T. chui* and *S. costatum*. The exponential equation (calculations of volume swept free) tends to underestimate feeding rates of growing cultures over the whole period, and overestimate cells removed on sinking cultures. Also, comparison of *S. costatum* feeding rates with that of the unicellular flagellates is difficult, due to the intrinsically variable nature of this difficult to count chained algae. Nevertheless, this should not alter the general conclusions derived from the data analysis.

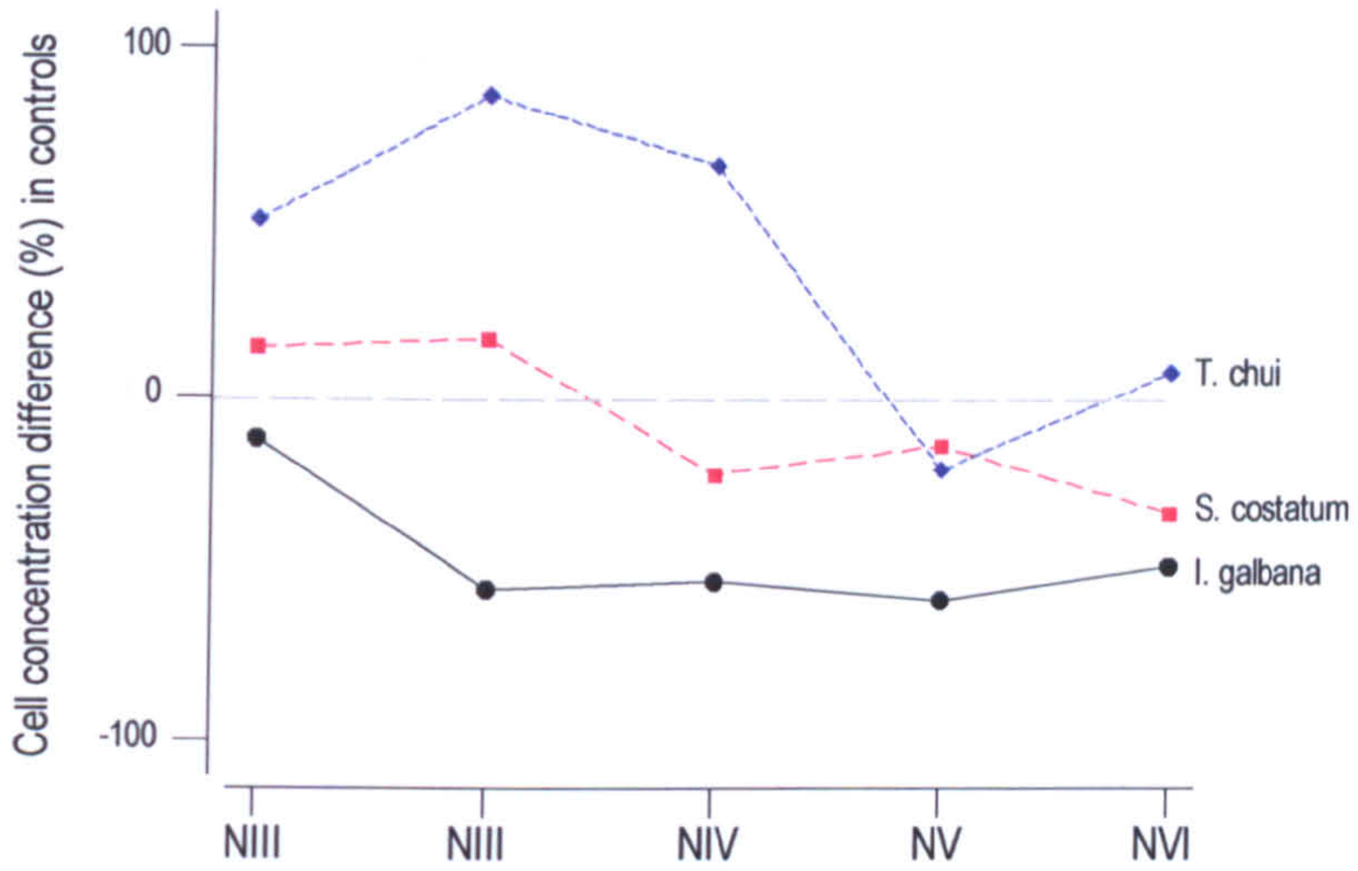


Figure III-12. Variation of algal density (%) in the control vessels of *P. pollicipes* larval feeding experiments, using algal monocultures of *I. galbana* (circles), *S. costatum* (squares), and *T. chui* (diamonds), at 100 cells μL^{-1}.

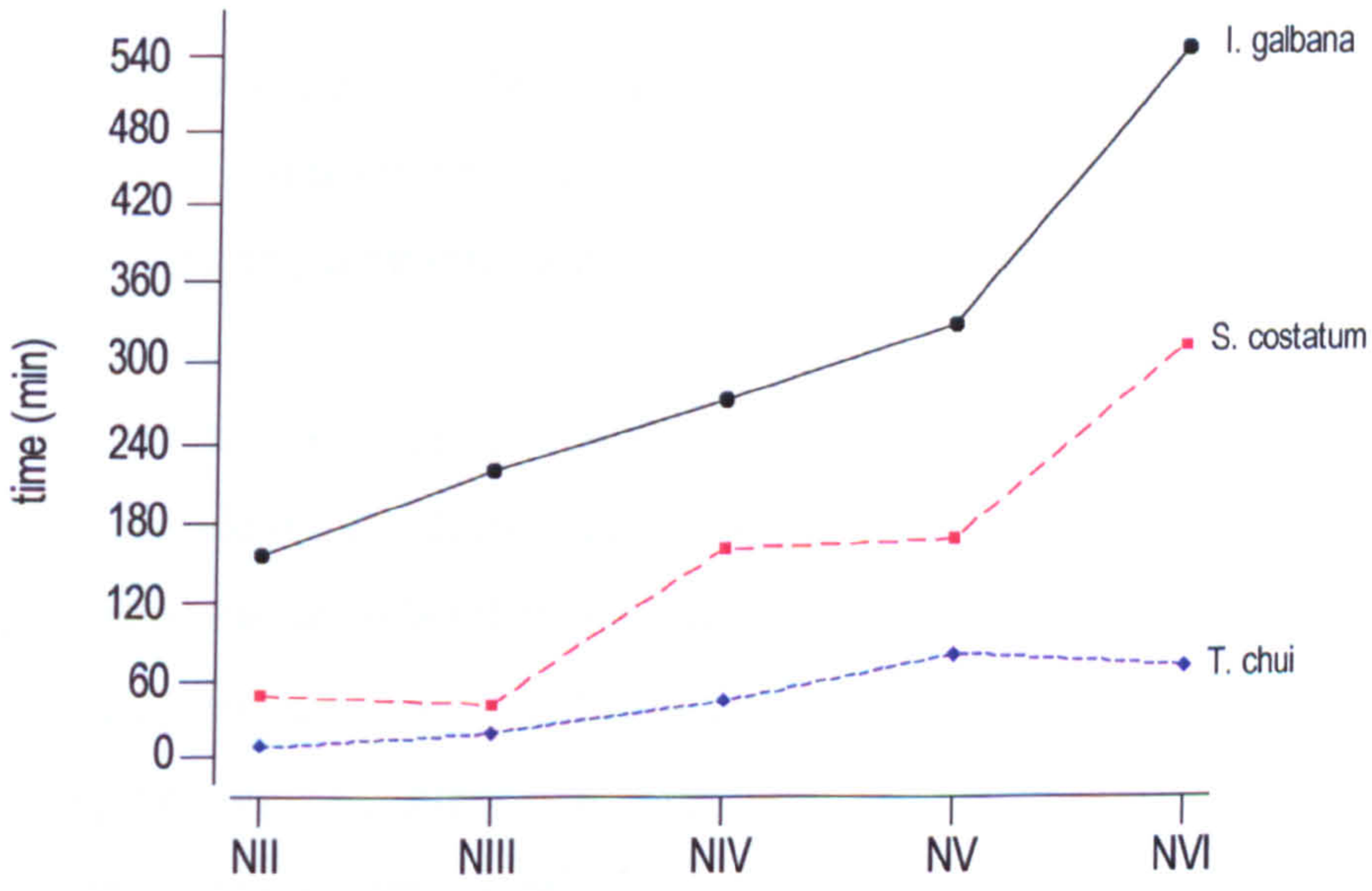


Figure III-13. Time taken to fill the gut of *P. pollicipes* larvae feeding on three algal monocultures at 100 cells μL^{-1}, of *I. galbana* (circles), *S. costatum* (squares), and *T. chui* (diamonds).

Walker *et al.* (1987) discussed the ability of balanoid larvae to accommodate to the food present in the suspension and the results on *P. pollicipes* nauplii are evidence of this behaviour. The volume swept by the larvae increased with the stage, which reflects that bigger larvae process larger amounts of water. Also, as they moult, larvae are able to handle larger phytoplankton cells and with lower ingestion rates they obtain relatively higher food volume.

It is interesting to note that the process of satiation slows with development, i.e. the time it takes to fill the larval gut is longer (Figure III-13). Balanoid larvae defecate every 15-20 minutes (Yule *pers. comm.)*. Observation of gut retention of fluorescent particles show that *P. pollicipes* larvae feeding on *I. galbana* produce the first faecal pellets after for 15-25 minutes (author's unpublished information). However, in the present experiment, only NIII feeding on *T. chui* gave values similar to this. Larvae feeding on *S. costatum* (highly variable as discussed above) and *I. galbana* took much longer to fill their guts. Considering that the observed ingestion rates were maximal at the given algal density, NVI larvae feeding on *I. galbana* would take almost 9 hours to be satiated. At the end of the experiments, condition of larvae was checked and all showed coloration of the gut with the respective algae derived from the suspension. Hence, (a) larvae are capable of retaining food in the gut for long periods and that the mechanism of defecation is decelerated or completely stopped accordingly to the food quality or (b) they do not utilise their maximal gut capacity, maintaining defecation at a regular rate with smaller faecal pellets.

Molares (*pers. comm.*) used *I. galbana* for rearing *P. pollicipes* at a density of 300 cells μL^{-1}, three times higher than that presented here. Hence, the low feeding rates reported on this alga might be due to the low density in which they were provided during the current study.

It is important to note that gut measurements were made on feeding larvae, reared with *R. reticulata.* For further analysis of satiation and defecation times, gut size should be measured on larval batches feeding in the respective algae and defecation times assessed according to each diet. Retention times may be an indicator of assimilation efficiency of a given alga, i.e. nutrient absorption may be greater if algae spend more time inside the digestive tube of the animal. However, in order for this to be ascertained, data on food value and its effects on development, mortality and recruitment rates would be required.

P. polymerus did not moult from NII when fed on *S. costatum* or *P. tricornutum,*

and *I. galbana* provided lethargic and weakened NIV larvae (Lewis 1975b), even when the proposed algal density was approximately $2.5E^6$ cells μL^{-1}. In fact, Lewis (*op cit.*) concluded that the excess concentration of algal cells might be disadvantageous as the toxic metabolites secreted by the algae can be lethal to the barnacle larvae. Without the use of antibiotics, it is recommended that, *in vitro*, food density be kept lower than 500 cells μL^{-1}.

Yule (1982) suggests that phytoplankton excretory products are identified by the nauplii as indicators of the density and quality of food present in the water. By means of a chemosensitive process, nauplii can alter their limb beat strategy in the presence of glycine, glutamic acid, glucose and sucrose (Yule 1982). Food presence in the larval gut seems to reinforce the capacity to distinguish edible particles once clearance rates are higher in the presence of food particles when compared to dissolved organic substances. Thus, in addition to the chemical stimuli, there is a mechanical stimulus of the algae, which is revealed by the difference in feeding effort on the three algae and the amount of ingested food. This could result from factors other than the differential shapes of the three tested algal, but control experiments would be required to determine what influences the naupliar preferences.

In order of preference, *P. pollicipes* larvae ingested larger amounts (μm^3) of the large flagellate *T. chui*, which concurs with the theory that temperate water nauplii prefer flagellates (Moyse 1963, Stone 1989). However, *P. pollicipes* also has the ability to feed on the chained diatom *S. costatum*, as demonstrated by the successful use of this alga by Kugele and Yule (1996). This is unsurprising considering that this barnacle inhabits colder waters characterised by temporary upwelling, and therefore is expected to be adapted to the high phytoplankton diversity of such areas.

P. tricornutum was not eaten by the metanauplii of *P. pollicipes*, probably due to the increase on the mesh formed by the interspace between the setae of naupliar limbs, which would not "sieve" the smaller cells (3-8 μm) of this diatom (Stone 1989). Nevertheless, several works have shown that algae used in feeding experiments in the laboratory must be supplied in much higher concentrations than that found in natural systems. For example, when Moyse (1963) tested *P. tricornutum* at a density 10 times higher than that of the present study, he obtained *Elminius modestus* cyprids on the twelfth day after hatching. On the other hand, *Balanus balanoides* never reached stage NIV, while *Chthamalus stellatus* and *Lepas anatifera* did not develop beyond stage NII when feeding on this diatom. Stone (1989) has also suggested than the sharp spines of

P. tricornutum may not be so palatable and irritate barnacle nauplii.

For the reasons given above, it is clear that feeding rates on different algal diets are only comparable if algal volume density is considered, and capture of different cells reflect the effort of feeding movements combined with the reward of obtaining energetic value.

Preliminary experiments showed *P. pollicipes* cultures were well maintained if regularly cleaned from accumulated moults and detritus (Kugele & Yule 1996). This procedure was more successful than avoiding total disturbance and leaving cultures without being cleaned. Thus, although aware of disturbance to nauplii caused by filtering and handling cultures, it was decided to clean cultures more often than usually stated in the literature (e.g. Stone 1988).

Extremely high algal densities tend to clog naupliar limbs, which prevent adequate feathering and increase the energy required for their movement, which in turn become vulnerable to the proliferation bacteria and consequent infection of the animals. However, the folding behaviour during the swimming-feeding mechanism allows nauplii to remove unwanted particles from the debris (Walker *et al.* 1987). Throughout this study, larvae were observed recovering from an apparent clogged state, with very little movement, to actively swimming nauplii and it is for this reason that sometimes there is an increase of larvae from one sampling day to the next.

The fastest development rate recorded in this experiment confirms that obtained by Kugele & Yule (1996) on a single laboratory culture. Previously, Molares (1994) had reported much slower rates, only obtaining the first cyprids after 23 days of hatching. Larval development rates based on laboratory studies often overestimate the duration of the larval planktonic period (Olson 1987 – probably due to lower nutritious value of aquaculture food provided in relation to that available in nature), and improvement of rearing methods *in vitru* permits more accurate assessment of these values.

The metamorphosis of larvae into metanauplii (NIV-NVI) is a delicate process, with mortality differing greatly between the tested diets. Considering the increase in size (present study), limb setation (Kugele & Yule 1996) and in the general morphological complexity of the larvae, nutritional requirements may change dramatically after NIII, and apparent suitable diets for rearing the early stages cease to be adequate for metanauplii and cyprid obtention.

After noting the ability of *P. pollicipes* larvae to feed on *S. costatum*, it was expected that higher survival results would be achieved with this diet. The same was

expected from cultures of *S. costatum* + *R. reticulata*, given that Kugele & Yule (1996) used the same diet when rearing all larval stages of this species. *S. costatum* on its own may not provide enough nutrients for survival of the last stages, although it is clear that it can be a food source for larvae for the period tested in the previous experiment (approx. 8 hours). Also, the condition of this algae was slightly variable, occasionally infected with bacteria (Budd, *pers. comm.*). Although effort was made to use the best quality algae, the changed batch of *S. costatum* following the Kugele & Yule (1996) study influenced the nutrional value of this diatom and consequently also the results from these diets.

R. reticulata provided as much nutrients as a mixed algal diet, or at least enough to rear all stages of *P. pollicipes* larvae, the difference being that in the latter, the density of *R. reticulata* was a fifth of that of the same alga in the first suspension. Thus, the food value of the 5 algae in a mixture is equivalent to the food value of the flagellate on its own. When combined with *S. costatum*, the lower concentration of *R. reticulata* was not compensated by the diatom and therefore the 2-algal mixture gave poorer results than the monoculture. Although development of larvae was faster with lower mortality using mixture of algae than using individual cultures (on *Mytilus edulis*, Bayne 1965), mixed diets are not necessarily advantageous when compared with unicellular algae (on *P. polymerus,* Lewis 1975b; on *E. modestus*, Stone 1989; on *Dreissena polymorpha*, Vanderploeg *et al.* 1996), for they depend on the individual value of the component algae. It is important to test the effect of mixed algae with barnacle nauplii feeding on natural plankton assemblages and this was undertaken for the first time by Turner *et al.* (2001). They concluded that *Balanus* cf. *crenatus* was mainly herbivourous, feeding less on ciliates and dinoflagellates, which corresponded to previous laboratory observations. Nevertheless, Le Vay *et al.* (2001) remarked that later stages of *E. modestus* may occasionally have a digestive enzymatic content characteristic of omnivorous zooplankton, which infers that these animals feed on heterotrophic organisms as well as phytoplankton.

In natural systems, the density of food appears to be compensated for by its diversity. In the laboratory, planktotrophics require much higher concentrations of food than those found in natural assemblages, showing greater selectivity of cultured foods than if fed on natural mixtures (Paulay *et al.* 1985; Baldwin & Newell 1995). Larvae release of *Semibalanus balanoides* was reported to be proportional to the food intake by the adults, which could be an adaptation to ensure an abundance of food for the planktotrophic larvae (Starr *el al.* 1991). Providing the release of larvae is synchronised

with spring phytoplankton blooms (Barnes 1962), it is essential to investigate feeding in the water column.

There is an apparent paucity of information on feeding of natural plankton by barnacle nauplii. Consequently, further studies using methods as direct as possible, capable of estimating ingestion rates *in situ*, would be invaluable. Availability of phytoplankton is seasonal and diversity and relative abundance may change with both temperature and time of day when samples are collected. Thus, multiple factors need to be taken into account when designing such experiments.

While data on natural plankton are not available, it is concluded that flagellates are a significant food source for scalpellomorpha larvae. *P. polymerus* grew better and healthier on a small flagellate (*Platymonas* sp.) until NIII and on a combined diet of small and larger flagellates (*Platymonas* sp. & *Prorocentrum micans*) after metanauplii. Although *Pollicipes* are able to ingest diatoms, monocultures of these algae did not provide enough nutrients for development. However, combining diatoms in a mixed diet of a highly nutritious "superior" algae (term proposed by Stone 1989), was successful on *P. polymerus* (Lewis 1975b) and *P. pollicipes* (Kugele & Yule 1996).

The geographical distribution of these animals, i.e. eastern Atlantic temperate waters, explains the results obtained in the laboratory. Generally, it is accepted that larvae released in warmer waters feed better on flagellates, while nauplii from cold waters harvest diatoms. *P. pollicipes* occupies an intermediate niche. Settling mostly in upwelling zones, where phytoplankton assemblages may shift within a period of 5 days (Joint *et al.* 2001), *Pollicipes* sp. are likely to encounter both flagellates and diatoms in the water column. While flagellates dominate waters during the autumn and winter mixing (Varella *el al.* 2005), diatom blooms characterise the continuum of cool water from spring to autumn. Meroplankton is mainly found in the frontal region between newly upwelled and coastal waters, on the boundaries of the upwelling core (Wing *et al.* 1998, Pineda & Lopez 2002), where flagellates are also likely to be found (Moita *et al.* 2003). Diatoms and chain-forming diatoms are more numerous in the upwelling core and in the outer side within mature oceanic waters, respectively (Abrantes 1988, Abrantes & Moita 1999, Olli *et al.* 2001).

Thus, it is understood that *Pollicipes* sp. are also able to feed on diatoms. In water mixing systems, diatoms can be the most abundant food source during a significant part of the *Pollicipes* life-cycle and it is logical that these animals have evolved to take advantage of them.

IV Feeding rates of *Elminius modestus* DARWIN 1854 nauplius II larvae in relation to mixed algal cultures

Numerous works have been carried out on *Elminius modestus* larval diet preferences. Within this study, comparative feeding rates between two contemporaneous algal species were calculated and ingestion dependence on algal densities was assessed. The flagellate *Rhinomonas reticulata* and chain-forming diatom *Skeletonema costatum* were offered as food to *Elminius modestus* stage II nauplii, at different cell densities and proportions. In order to maintain constant experimental conditions, a continuously rotating wheel immersed in a temperature-controlled tank was used. The diatom was preferentially ingested, even when the flagellate was present in relatively higher cell volume densities. Larval gut volume and maximal ingestion rates were in accordance with defecation rates known for this species. Selective feeding behaviour of these barnacle larvae may represent an ecological advantage in an environment of patchy phytoplankton assemblages.

IV.1. Introduction

Since Knight-Jones & Waugh (1949) described *Elminius modestus* larvae, this has been one of the most studied barnacles, together with *Semibalanus balanoides*, in the area of larval physiology. The long reproductive season and continuous availability of *E. modestus* larvae (O'Riordan & Murphy 2000) have enabled researchers to undertake laboratory experiments virtually throughout the year.

Phytoplankton monocultures have been used to feed *E. modestus* nauplii and *Phaeodactylum tricornutum*, *Ditylum*, *Skeletonema*, *Asterionella*, *Thalassiosira weisflogii*, *Chrysochromulina ericina*, *Isochrysis galbana*, *Rhodomonas* sp. (Wisely 1959, Moyse 1963, Stone 1988, 1989) have produced high rates of development and survival. Conversely, when fed cultures of *Dunaliella primolecta*, *Micromonas pusilla*, *Prymnesium parvum*, *Amphidinium* sp., *Chlorella stigmatophora*, *Pyramimonas grossii*, *Cryptomonas* sp., and *Hemiselmis rufescens* the larvae did not develop beyond NII-NIV. When fed *Chaetoceros calcitrans* and *Prorocentrum micans*, the larvae passed stage IV but exhibited deformities of the ventral thoracic process and were

smaller in size.

The idea that more natural multi-species algal cultures might produce better survival of reared invertebrate larvae (*Mytilus edulis*, Bayne 1965; copepods, Nassogne 1970; *Pecten maximus*, Gruffydd & Beaumont 1972; *Ostrea edulis*, Helm 1977) led researchers to test several mixed cultures in rearing larvae. However, Stone (1988) found that a monoculture of *I. galbana* in the *Elminius modestus* earlier larval stages followed by *Rhodomonas* sp. after 9 days from hatching produced faster rates of development, better survival and larger larval size, than a mixture of both algae.

Inadequacy of a diet was often explained by the toxic nature of the algae, and such toxicity has already been shown for other organisms (copepods, mysids, fish, see Moyse 1963). Galinidi (2001) analysed the chemical effect of macro-algal exuviates on *E. modestus* and she found that crude extracts from *Coralina*, *Cladophora* and *Lithothamnia* were highly toxic for *E. modestus* NII larvae, besides reducing the ingestion rate and the cirral activity of the adults.

Taste, and even the strength of the cell wall have been thought to influence ingestion of algae by copepods (Gauld 1951, Petipa 1959 in review by Yule 1982), but size and shape of food cells have been generally considered as the major factors controlling selective feeding by planktonic herbivores.

Nauplii have to be able to manipulate the algae in order to ingest them, i.e. while swimming, the three thoracic appendages move the water surrounding the animal's body which contains food particles. Because localised currents are generated during the swimming, in this aquatic environment there has to be a mechanism to retain food particles near the labrum and the midline groove that leads to the mouth. On the recovery stroke of the antennae, cells are trapped between the limbs, and the body, then forced towards the labrum by the mandibles to be trapped in labral secretions and finally pushed by the limb gnatobases inside the mouth. The labral secretions increases viscosity in the ventral area but cannot discriminate between particle sizes. Stone (*E. modestus*, 1989) believed that such discrimination is determined by the antennae intersetule distances, which vary between barnacle species. She showed that the narrower the space between the setules, the smaller the algal cells nauplii are able to feed on.

Another type of feeding behaviour of *E. modestus* larvae has been reported by Wisely (1959) and Crisp (in Walker *et al.* 1987). These authors observed that nauplii were able to seize fertilised eggs (mollusc and annelid) by means of the "masticatory

spines at the mandibular bases and carried them round while swimming (...), providing the material was sufficiently fluid, it was sucked in by pumping movements of the oesophagus". Other types of food of animal origin were used to enrich phytoplankton diets, and although there is little consistent scientific work on animal predation by *E. modestus*, Ngamphongsai (2000) and Le Vay *et al.* (2001) have suggested, by analysing the digestive enzymatic activity, that the final naupliar stages may well be omnivorous.

Hence, *E. modestus* is receptive to a wide range of diets and this corresponds with its extensive geographical distribution in intermediate latitudes. Although much work has been undertaken to explain food selectivity, researchers have given more attention to the quality of diets, while quantity of food has only been tested in monocultures. Little is known about the feeding process of barnacle larvae in relation to the contemporaneous presence of two edible algae. In the natural environment, relative density between groups of phytoplankton changes seasonally (Bainbridge 1953, Legendre & Demers 1984, Mackas *et al.* 1985). Furthermore, algal species exist in the water column in patches, which results from binary fission and leads to population growth (Dagg 1977).

Is the feeding mechanism automatic or are barnacle nauplii adapted to spend energy discriminating between certain foods, thus ingesting those of higher nutritional value in greater proportion to their environmental availability?

Harms (1988) suggested that without food, barnacle nauplii do not moult beyond larval stage II and several authors have analysed the effect of starvation immediately after hatching on development and survival rates (see review in Lang & Marcy 1982, *Balanus improvisus*). Though it has been shown that survival potential decreases significantly in proportion to the duration of starvation (*Balanus amphitrite*, Qiu *et al.* 1997, Dessai & Anil 2002) naupliar feeding behaviour has not be addressed relatively to initial lack of food. The present study, investigates whether starved nauplii show any modification of the clearance and ingestion rates in relation to fed ones.

IV.2. METHODOLOGY

IV-2.1 Preparation of larvae

Preceding the first set of observations, NI larvae were released from the adult *E. modestus* (see General Methodology) and maintained for 12-14 hours in a culture of *Rhinomonas reticulata* at a density of 100 cells μL^{-1}. The nauplii were stocked at a density of 1-3 animals ml^{-1}. The cultures were gently bubbled with compressed air, and kept in the temperature controlled tank at 17.5 ± 0.5°C in constant light. For the second set of observations, NI larvae were treated exactly as above except they were maintained in UV irradiated and fine filtered (0.2μm) seawater without food. Only actively swimming NII larvae were selected for all experimental observations.

IV-2.2 Larval feeding experiments

Algal suspensions were prepared with different proportions of the flagellate *Rhinomonas reticulata* and the diatom *Skeletonema costatum*. One hundred actively swimming *E. modestus* larvae were selected and placed into 120 ml of algal culture in glass bottles. The bottles were then incubated on a rotating wheel immersed in a temperature-controlled tank at 17.5 ± 0.5°C. For each experimental culture, control bottles were set up with the same algal concentrations but without larvae.

Three 1ml samples were taken from each bottle and fixed with 100 μL of Lugol's solution at the beginning and end of the experiment. Experiments were run for measured time intervals of approximately 24hrs with a 12h Light:Dark cycle. The algal densities were estimated by counting individual cells on a modified Fuchs-Rhosenthal haemocytometer. Clearance and ingestion rates were calculated as described in Chapter III.

IV.3. RESULTS

IV-3.1 Larval measurements of *Elminius modestus*

The methods used to measured total length (L), the greatest width (W), the cephalic shield length (CSL), the total gut length (GL) and the greatest gut width (GW) of *Elminius modestus* were described in Chapter II General Methodology.

The results in Table IV-14 show the linear dimensions of the larval stages, indicating the constant increase in size of the larvae through development. Ranges for virtually every parameter overlap considerably between adjacent stages. Such is particularly true for NI and NII larvae, where the longest and widest nauplii of either stage measured approximately the same. Other than for the length of NI, stage V nauplii appeared the most variable over most dimensions.

Table IV-14. Measurements of total length, greatest width and cephalic shield length of *E. modestus* larvae.

	total length (µm)				greatest width (µm)				carapace length (µm)			
stage	n	Mean	StDev	Range	n	Mean	StDev	Range	n	Mean	StDev	Range
Ni	15	370	45	224-408	15	154	16	102-173				
Nii	25	397	19	357-428	22	147	14	122-173				
Niii	36	435	17	380-460	36	194	17	140-235				
Niv	41	471	19	400-500	43	230	21	200-320	42	300	19	200-330
Nv	35	511	36	380-560	32	262	39	180-350	36	351	34	280-450
Nvi	5	570	10	560-580	20	351	42	230-430	32	472	44	340-520
Cyprid	9	214	12	200-230	9	534	11	520-550				

Bonferroni pairwise comparisons procedure for fit test was significant at all levels ($F_{df=84[2,140]} = 12.64$, $p < 0.001$) indicating that a multiple regression function using L and W as descriptors was appropriate to predict larval stages. The regression equation was significant ($F_{df=2,140} = 291.96$, $p < 0.001$) and the relation between stage and L & W of *E. modestus* nauplii (NI-NVI) and respective coefficients are shown on Table IV-15.

Table IV-15. Multiple regression function between total length and greatest width (predictors) and naupliar stage (nauplii I-VI are 1-6, response) of *E. modestus*.

stage = -3.68 + 0.00935 L + 0.0138 W

Predictor	Coef	SE Coef	T	p-value
Constant	-3.6759	0.4891	-7.52	<0.001
Length	0.009352	0.001744	5.36	<0.001
Carapace Width	0.013792	0.001909	7.23	<0.001

NVI larvae showed bending of the ventral thoracic process, which might prevent accurate measurement of L. The flexure is preparative for the moult to cyprid when all the body eventually fits insides the folded carapace. In this case, W and CSL can be used to discriminate metanaupliar stages, as shown by the Bonferroni's fit test significant at all levels ($F_{df=53[2,89]} = 2.21$, $p < 0.007$). The resultant regression function was significant ($F_{df=2,89} = 251.90$, $p < 0.001$) and respective coefficients are shown on Table IV-16

Table IV-17 shows the mid-gut dimensions for all naupliar stages but NI, which lacks a functional gut. Noticeably, the average length and width for stage II & III vary very little. A steady increase in average occurs from NIII – NV but then both length and width decrease, on average, at NVI.

The average mid-gut volume for *E.modestus* naupliii was calculated from length and width (see Table IV-17) assuming the shape of the gut approximates that of an ellipsoid (Table IV-18). Gut volume clearly does not increase consistently with development, hence larval size. NII & NIII have the same gut volume but that increases by a factor of 5 on moult to NIV. The volume then almost doubles from NIV to NV before decreasing again at NVI.

Table IV-16. Multiple regression function between greatest width and carapace shield length (predictors) and metanaupliar stage (nauplii IV-VI are 4-6, response) of *E. modestus.*

stage = 1.23+0.00347 W + 0.00728 CSL

Predictor	Coef	SE Coef	T	p-value
Constant	1.2276	0.1645	7.46	<0.001
Length	0.0034745	0.0009566	3.63	<0.001
Carapace Width	0.0072760	0.0006906	10.54	<0.001

Table IV-17. Measurements of total gut length and width of naupliar *E. modestus* larvae (nauplii II-VI)

	gut total length (µm)				gut greatest width (µm)			
stage	n	Mean	StDev	Range	n	Mean	StDev	Range
Nii	25	125	13	102-143	23	55	9	31-71
Niii	35	127	24	70-180	34	51	11	30-71
Niv	38	176	47	100-270	38	103	41	40-180
Nv	26	265	42	120-290	26	126	20	80-170
Nvi	24	260	19	220-300	20	98	11	80-120

Table IV-18. The means, standard deviation and range of larval gut volume (10^5 µm^3) of actively swimming *E. modestus* larvae considering the total length and greatest width of an ellipsoidal shaped gut.

larval stage	Gut volume (x10^5 µm^3)			
	n	Mean	StDev	Range
Nii	23	2.1	0.7	0.7 – 3.5
Niii	34	1.8	0.8	0.5 – 3.6
Niv	37	12.1	8.3	1.0 – 28.8
Nv	26	22.7	7.4	6.3 – 36.2
Nvi	20	13.3	2.7	8.4 – 17.3

IV-3.2 Feeding rates of *Elminius modestus* NII on *Skeletonema costatum* and *Rhinomonas reticulata*

Throughout this experiment total cell concentrations for each suspension were fixed at a convenient range of cell density providing a range of dietary proportions of *S.costatum* and *R.reticulata* (cell:cell density). As a consequence, *S. costatum* cells were always present at much lower cell volume concentrations than were, the much larger, *R. reticulata* cells.

Figure IV-14 shows the clearance rates of *E. modestus* nauplii while feeding on the cultures of *R. reticulata* and *S. costatum.* The clearance rates are quite variable, showing very little difference between fed (mean = 13.2 ± 7.3 μL animal^{-1} hour^{-1}) and unfed larvae (mean = 12.6 ± 6.9 μL animal^{-1} hour^{-1}; two sample t-test = 0.28, p = 0.778 for df = 51). Such lack of difference proved a common feature throughout the results hence distinctions between fed and unfed larvae will not be considered further. Despite the fact that there was no significant correlation (r = 0.04, p = 0.774, df = 50) between overall (*S. costatum* and *R. reticulata* combined) clearance rates and cell volume density, a significant decrease in clearance rate was evident when *S. costatum* was present in proportions below 50% (r = -0.449 p = 0.024 df = 23) but not when the alga was present at higher proportions in the cultures (r = 0.181 p = 0.357 df = 26, see Figure IV-14).

Clearance rate declines significantly (r = -0.395 p = 0.007, df = 43) with cell density to around 400 cells uL^{-1} (Figure IV-15). Thereafter, clearance rates remain around 10 μL animal^{-1} hour^{-1} or even start to rise at the highest concentrations. However, all concentrations above 400 cells μL^{-1} represented the highest proportions of *S. costatum* (by volume), which on the other hand only represented a small proportion of the total volume of cells available. The larger *R. reticulata*, even if present at small cell densities in comparison to *S. costatum*, generally constituted the great proportion of the total volume. At low cell densities of *R. reticulata*, hence high volume density, clearance declines as cell density increases (regression estimates a slope of 0.05 ± 0.02 μL cell^{-1} μL^{-1}). Above 400 cells μL^{-1}, the total cell volume density is low because most of the cells are *S. costatum* and clearance rates remain fairly stable.

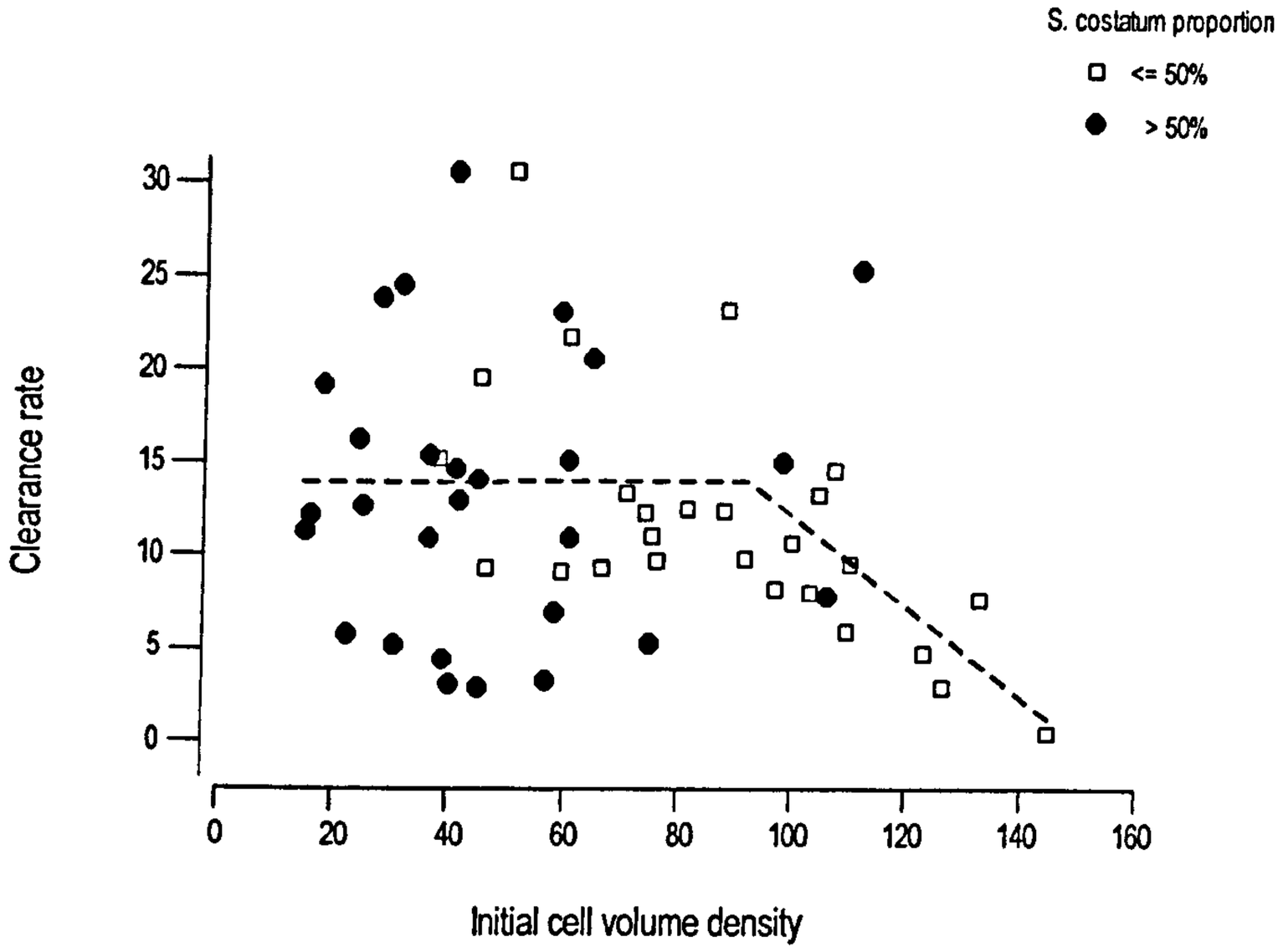


Figure IV-14. Clearance rates (µL animal^{-1} hour^{-1}) of *E. modestus* nauplii II feeding on mixed *S. costatum* and *R. reticulata*. The abscissa is cell volume density (x10^{3}µm^3 µL^{-1}).

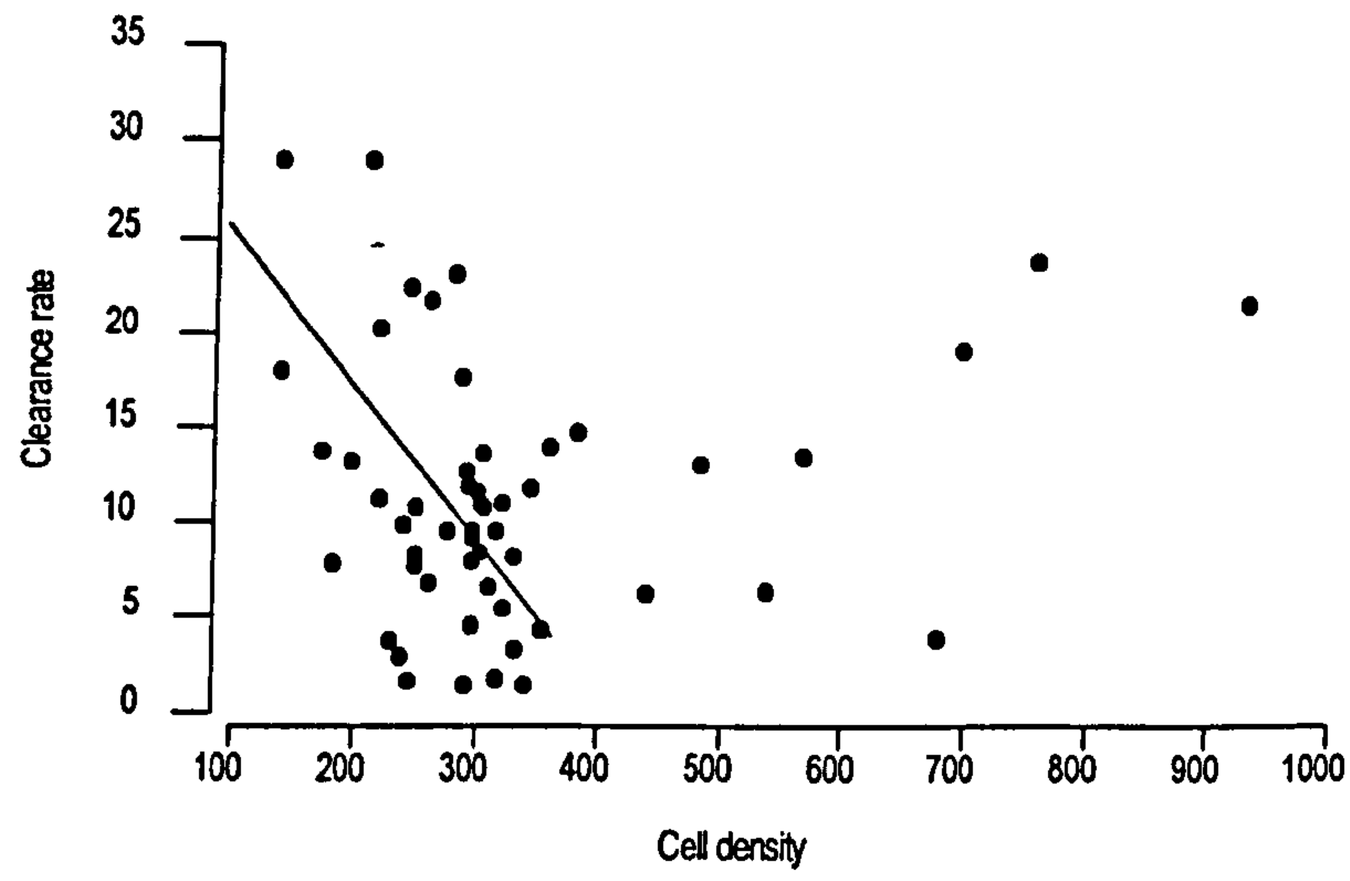


Figure IV-15. Over all clearance rates (*S.costatum* and *R.reticulata* combined) for *E. modestus* nauplii II versus cell density of mixed algal cultures. Line fitted by least squares regression of data below 400 cells µL^{-1}.

Individual clearance rates for stage II nauplii, feeding on mixed cultures of *R. reticulata* and *S. costatum*, relative to cell volume density are illustrated in Figure IV-16. The distinction is again made between high and low proportions of *S. costatum* in the diet. At high *S. costatum* proportions, clearance rates are very variable and there is even a suggestion in the results that clearance is highest at lowest cell volume densities, declining afterwards to a stable level around 15-16 uL animal^{-1} hour^{-1}. The variability is such that no relationship is significant and the impression may be driven by the particularly high clearance rate (60 µL animal^{-1} hour^{-1}) seen at one of the lowest cell volume concentrations. At densities above 70x10^3 µm^3 µL^{-1} (low proportions of *S. costatum*) clearance rates decline with increasing food availability.

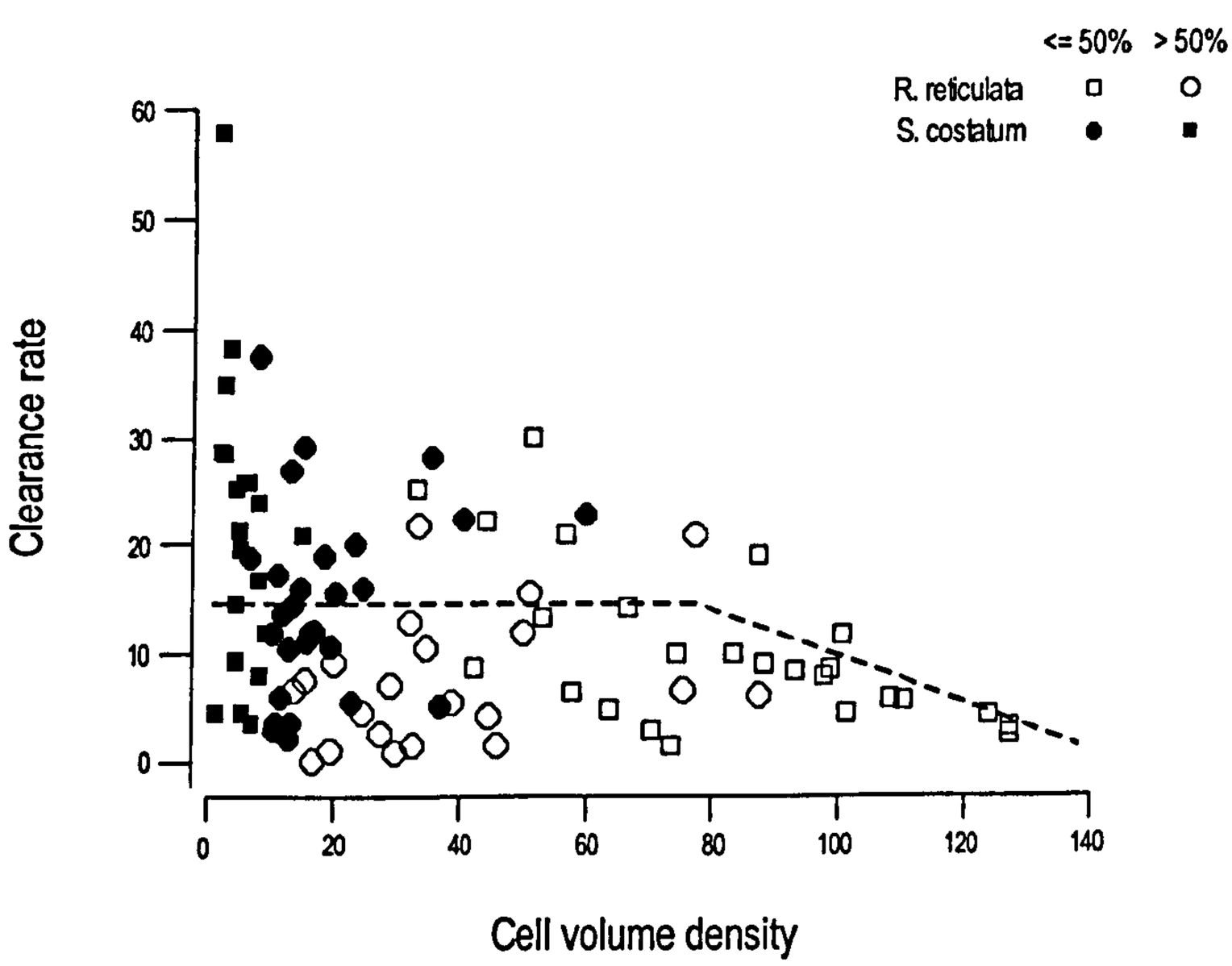


Figure IV-16. Individual clearance rates (µL animal^{-1} hour^{-1}) for *E. modestus* nauplii II feeding on *R. reticulata* and *S. costatum* in mixed culture. The abscissa is cell volume density (x10^{3}µm^3 µL^{-1}) and lines fitted by eye (see text).

Figure IV-17 shows the total ingestion rate of *E. modestus* stage II nauplii feeding on the mixed algal cultures. Ingestion is expressed as cell volume ingested and cell density as cell volume density. Once again the problematic of *S. costatum* proportions in the initial cultures is highlighted. For *S. costatum* proportions above 50%, a significant positive correlation ($r = 0.554$, $df = 25$, $p = 0.003$) indicated an increase in ingestion rate with cell volume concentration. No significant correlation was evident ($r = -0.189$, $df = 23$, $p = 0.366$) at low proportions of *S. costatum* where the mean ingestion rate ($8.02 \pm 3.8 \times 10^5 \mu m^3$ animal^{-1} hour^{-1}) is indicated on Figure IV-17. Thus, the total cell volume ingestion rate for NII *E. modestus* appears to increase with cell volume density before levelling off at a maximal, if quite variable rate, somewhere in the vicinity of $40 - 90 \times 10^3 \mu m^3\ \mu L^{-1}$.

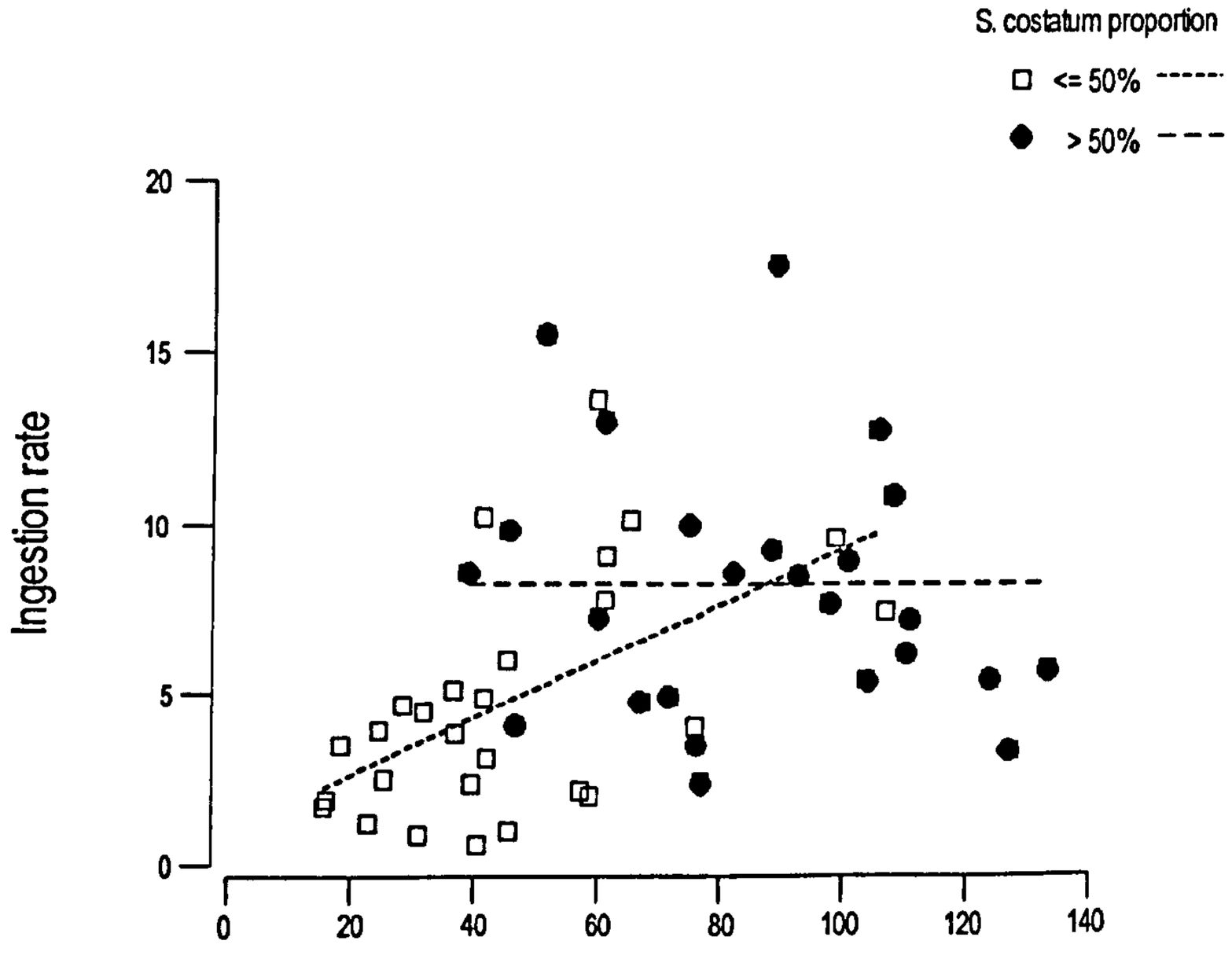


Figure IV-17. Ingestion rates ($x10^5 \mu m^3$ animal^{-1} hour^{-1}) of *E. modestus* nauplii II feeding on mixed cultures of *S. costatum* and *R. reticulata*. The abscissa is cell volume density ($x10^3 \mu m^3\ \mu L^{-1}$). Lines represent a least squares regression fit for *S. costatum* at low proportions (short dash ≤50%) and the mean ingestion rate for *S.costatum* at high proportions (long dash >50%).

The individual ingestion rates by nauplii are plotted against cell volume density (individual species) in Figure IV-18. Irrespective of proportion, the cell volume ingestion rate of *S. costatum* cells by the nauplii increases with cell volume density ($r = 0.853$, $df = 46$, $p < 0.001$). By contrast, the cell volume ingestion rate on *R. reticulata* by nauplii parallels the total cell volume ingestion rates, positively correlated ($r = 0.678$, $df = 20$, $p = 0.001$) with cell volume density at high proportions of *S. costatum* yet showing no significant correlation ($r = -0.187$, $df = 26$, $p = 0.372$) when *R. reticulata* proportions were high.

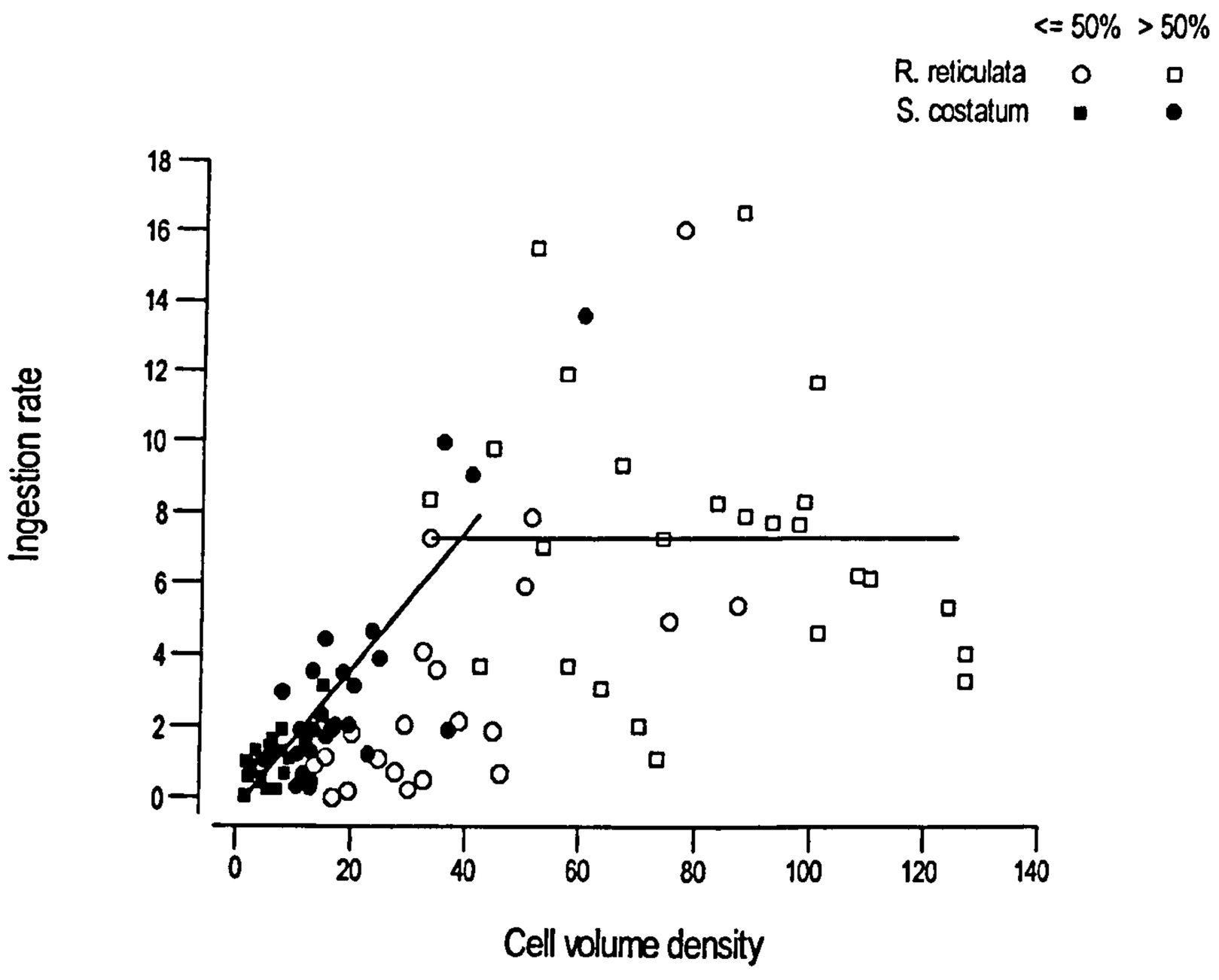


Figure IV-18. Individual ingestion rates ($x10^5 \mu m^3$ animal^{-1} hour^{-1}) by *E. modestus* nauplii II on mixed cultures of *R. reticulata* and *S. costatum*. The abscissa is cell volume density ($x10^3 \mu m^3$ μL^{-1}). Lines fitted by eye.

The regression analysis is described in Table IV-19 as well as the comparison between the change in ingestion rate over cell volume concentration for larvae feeding on *S. costatum* and for larvae feeding on *R. reticulata* at high *S. costatum* proportions.

The rate of change of larval ingestion rate on *S. costatum* is over 50% greater than that on *R. reticulata*. The difference was significant (Table IV-19 (b), interaction

term). The intercepts are both negative but neither are significantly different from zero, so great is the variability. Negative intercepts would imply a positive intersection with the cell volume density axis. For *S. costatum* in mixed culture, that positive intersection would be approximately at 1950 μm^3 μL^{-1} (~40 cells ul^{-1}) and for *R. reticulata* at 11100 μm^3 μL^{-1} (also ~ 40 cells ul^{-1}). The difference in intercept and the impression from Figure IV-18 suggest that *S. costatum* may be ingested at a faster rate than *R. reticulata* when cell volume ingestion rates are less than maximal and both species are contemporaneously available in the water.

Table IV-19. Analysis of individual ingestion rates for *E. modestus* nauplii II feeding on *R. reticulata* and *S. costatum* in mixed culture. Regressions of ingestion on *S. costatum* with cell volume density and *R. reticulata* (at high *S. costatum* proportions only) with cell volume density.

(a) Regression slopes (μm^3 $animal^{-1}$ $hour^{-1}$ $(\mu m^3 \mu L^{-1})^{-1}$) and intercepts ($\mu m^3$ $animal^{-1}$ $hour^{-1}$)

Species	Slope	±SE	Intecept	± SE
S.costatum	18.7	1.8	-36917	31276
R.reticulata	12.2	3.0	-136181	128540

(b) Analysis of variance of algal species versus initial cell volume density in mixed cultures.

Source	DF	Seq SS	Adj SS	Seq MS	F	p
Algal Species	1	1.613×10^{11}	3.145×10^{10}	1.613×10^{11}	4.41	0.040
Volume density	1	3.420×10^{12}	3.614×10^{12}	3.412×10^{12}	93.41	<0.001
Interaction	1	1.979×10^{11}	1.979×10^{11}	1.979×10^{11}	5.41	0.023
Error	66	2.416×10^{12}	2.416×10^{12}	3.661×10^{10}		
Total	69	6.195×10^{12}				

Considering the proportion (by cell volume) of *S.costatum* and *R.reticulata* in the diet relative to the proportions (again by cell volume) of both algae in the mixed culture, the results lie close to the 1:1 line for *R.reticulata* when close to zero% availability and for *S.costatum* when close to 100% availability, i.e., proportion of *S. costatum* in diet is higher relatively to that present in the environment (Figure IV-19).

Trend lines have been fitted by least squares regression ($r = \pm 0.792$, $p < 0.001$, for both) and clearly show that for most of the range of availability *E. modestus* larvae ingest *S. costatum* cells (by volume) at a higher proportion than their availability. Conversely, the trend also shows that *E. modestus* consume *R. reticulata* cells (by volume) in lower proportions to their availability. It would appear that *E. modestus* larvae show a fixed 'preference' for the diatom over the flagellate despite the large difference in cell volume between them.

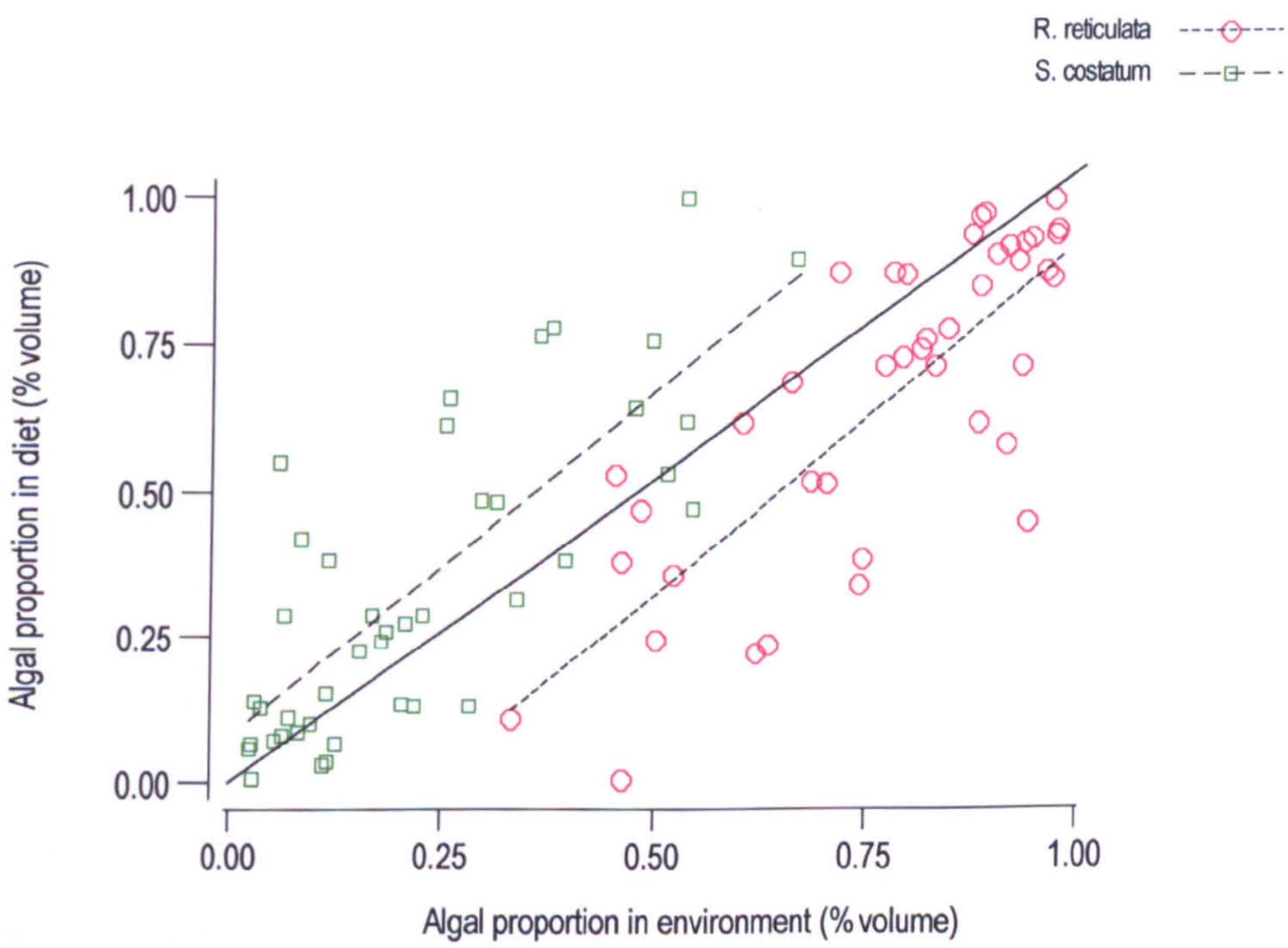


Figure IV-19. Proportions (by volume) of each alga (*R. reticulata* and *S. costatum*) ingested by *E. modestus* nauplii II per unit time against the proportion of that alga available in the water. The solid line represents a 1:1 proportion (random selection). The dotted lines represent linear trend lines fitted for each alga. NB: monocultures representing either 0 or 1 have been omitted.

IV.4. Discussion

The results of the current study clearly showed that *E. modestus* NII have higher cell volume ingestion rates on *S. costatum* than on *R. reticulata*, when simultaneously presented with both algae in suspension. Even when the flagellate was present in much higher cell volume densities and relative proportions, the diatom was still preferentially ingested. The interpretation of the results for an experiment such as this requires careful observation of the algal cells and assumes that the cultures of both algae are equally 'healthy'. Initially, *S. costatum* cultures were infected by bacteria and proved toxic to the larvae. All those experiments had to be repeated with 'healthier' *Skeletonema* cultures. Although it is known that *E. modestus* NII larvae have an optimum clearance rate at such cell concentrations (Yule 1982), in this study it was not established whether if, in conjunction with another alga, a lower final concentration would have been just as adequate.

When Lang and Marcy (1982) and Harms (1988) investigated starvation resistance of *Balanus improvisus*, *E. modestus* and *Semibalanus balanoides* they suggested that survival potential may decrease significantly if larvae are left without food for as little as 24 hours after hatching, i.e., the energy loss and deceleration of the development processes are irreversible. In the current study, nauplii were left without food for a shorter period (12-14 hours) and showed no differences in feeding rates or selectivity from those of larvae continuously fed from hatch. The starved larvae were not able to, or did not need to, increase feeding rates to offset the energy loss occuring through the starvation period. However, the effect of a period of starvation might differ between species. Larval release in certain species has been highly correlated with diatom blooms (*S. balanoides*, Barnes 1962, Starr *et al.* 1991), while others seem to be strongly related to temperature and salinity at the time of release (*Balanus improvisus*; Lang & Ackenhusen-Johns 1981). Species with larval released coupled to phytoplankton abundance may well suffer more from a period of starvation than those coupled to other environmental factors. *E. modestus* release larvae continuously through the year into quite disparate food densities and may therefore carry greater yolk reserves at NII than species with larval release tightly coupled to food abundance (e.g. *S. balanoides*).

The need to use of cell volume rather than cell number to define the density of the food suspension is clearly identified in the analysis of the current results. The differences in cell size between *R. reticulata* and *S. costatum* (i.e. mean cell volumes of 370 and 64 μm^3, respectively) resulted in highly variable results when presented together to the larvae. Considerable reflection was required during analysis to extricate the underlying trends in the data with regard to total feeding rates and those on individual species within the mixtures. An algal volume of 85000 μm^3 corresponds to a *R. reticulata* density of 230 cells μL^{-1} and to 1328 cells μL^{-1} of *S. costatum*. These cell densities are much higher than those used in aquaculture of commercial meroplankton and than those considered as the density of natural phytoplankton assemblages, usual maxima of 0.5 cells μL^{-1} for diatoms and 50 cells μL^{-1} for flagellates (see Bainbridge 1953). The use of higher than natural densities of cells in laboratory work is expected: many laboratory cultures of algae result in smaller than natural cell sizes because laboratory cultures are usually maintained in exponential growth phase.

S. costatum cells, though smaller than those of *R. reticulata* do form chains up to 50 µm and single cells are rare in culture. These chains can often clog limbs of the nauplii when present at very high densities. However, at a low concentration, these long chains might be easier to capture than the larger but single cells of *R. reticulata*. The larva does not have to capture cells one by one; on the contrary, if it comes across a long chain it might easily grasp it under its ventral side, next to the labrum, and eventually eat it, which, at such low densities, would make a greater difference to the cell volume variation. Yule (1982) observed *E. modestus* NII larvae did not feed differentially on *S. costatum* chains up to 5 cells (~20 µm long). However, the algal batches used during the current experiment contained a relatively high density of long chains (> 5) and the larvae might have fed more efficiently on those. Unfortunately, the number of cells per chain was not assessed during the algal counting for the current data.

Estimating ingestion rates on *R. reticulata* and on *S. costatum* according to Table IV-19 (a), and that the mean volume of NII *E. modestus* larval gut is approximately $2.1x10^5 \mu m^3$ (– see Larval Measurements, Table IV-18) it is possible to calculate the time of larval defecation according to algal density. Considering suspensions contain only one algal species at a density of approximately 200 cells μL^{-1}, animals would be satiated in 16 minutes with *R. reticulata*, while it would take over an hour to fill their guts with *S. costatum*. At equal cell volume densities ($13x10^3$ –

$13x10^4$ μm^3 μL^{-1}), however, *S. costatum* will fill a nauplius' gut in about 10-60% of the time that *R. reticulata* will. Defecation rates in *E. modestus* NII larvae feeding on *S. costatum* vary from 1 to 5 pellets $nauplius^{-1}$ h^{-1}, largely dependent on algal concentration (Yule 1982). Gut filling times for nauplii at maximal ingestion rates from the current results lie within this defecation rate range.

Moyse (1963), using *S. costatum* as the diet, obtained *E. modestus* cyprids only 5 days after larval hatching, while Stone (1989), using *Rhodomonas* as the diet, (probably *R. reticulata*), reared the larvae but achieved lower development rates, which suggests a differential nutritional values of these algae. In order to determine assimilation efficiency, defecation rate of *E. modestus* NII larvae needs to be measured as well as the chemical composition of each alga. From this, the nutritional contribution of each algae could be determined

Thus, in order to address community interactions in food webs of planktonic organisms, future reseach projects should aim to study the feeding response of barnacle nauplii in multialgal assemblages, combined with chemical composition analysis of the phytoplankton and metabolic requirements of the feeders. Nauplii might select certain algae likely to provide more nutrients than others, obtaining a higher return of the energy spent as swimming planktotrophic larvae, which, if the energy cost of that selection is sufficiently low, would represent an advantage in relation to non-selective species.

V Swimming activity of barnacle larvae: response to food

Speeds of *Pollicipes pollicipes* metanauplii (nauplius IV to VI) were measured by timing continuously swimming larvae towards a light source. Preliminary experiments with nauplii IV showed a significant decrease of speed with repeated measurement over the same larva. Swimming response of all developmental stages of *Elminius modestus* to food presence and starvation was recorded using a VCR and video camera attached to a dissecting microscope with a $1mm^2$ grid. Maximal speeds increased with stage from nauplius I to V, yet the rate of increase diminished from nauplii to metanauplii. Nauplii II feeding on *Rhinomonas reticulata* (100 cells μL^{-1}) were 20% faster than non-feeding larvae, while there were no significant differences between speeds of nauplii VI feeding on the flagellate and a mixture of algae. Increase of larval speeds during earlier metamorphosis was associated with dispersion and nutritional needs of initial larval stages, suggesting a dramatic physiological change during the metanaupliar moult, which was confirmed by detailed observations on larval behaviour. Cyprid swimming speeds ranged from 1.76 – 64 mm sec^{-1}. These results were compared with published speeds of planktotrophic and lecithotrophic cirripedes and larval functionality discussed within the scope of water viscosity. Swimming speeds and behaviour reflect larval requirements of dispersal, feeding, energetic efficiency and ultimately settlement.

V.1. INTRODUCTION

For sessile benthic animals, the production of planktonic larvae is a vital mechanism for dispersal. Hydrodynamic processes, like tidal currents and eddy diffusion, disperse the propagules from the vicinity of the parents, maintaining genetic diversity and increasing the probability of colonising empty habitat. It is also said that differences in ecology between life history stages reduces competition between adults and their larvae thereby increasing recruitment through greater numbers of larvae initially and potentially better larval survival (McEdward 1995). Neither hypothesis has been actively tested.

In intertidal species, strategies of retention or recovery of these larvae on the coastal shelf and thence the shoreline have been investigated; these investigations centre on animal abundance, distribution and migration, along with water motion, transport and hydrodynamic processes (reviews *in* de Wolf 1973, Chia & Buckland-Nicks 1984 and more recently, Price 1989, krill and algal patches; Saiz 1994, *Acartia tonsa*, food and turbulence; Abelson & Denny 1997, settlement under different flow

regimes; Clough *et al.* 1997, hydrography on meroplankton geographical distribution; Anastasia *et al.* 1998, tagging larvae and dispersal; van Duren 2000, kinematics of *Temora longicornis*; Helfrich & Pineda 2002, Shanks *et al* 2003,larval transport).

As vertical mixing is a relative continuous process, larvae have to swim strongly enough to reach and maintain an adequate position in the water column e.g. to feed, to avoid predation or return to the shore (Macho *et al.* 2005). When larvae stop swimming, they either sink or float, depending on their density, and become susceptible to the vertical mixing. Active swimming allows larvae to recover from unfavourable mixing out of position and return to areas of high food availability or potential settlement substrata. Horizontal migration of various animals into higher concentrations of phytoplankton has been demonstrated in the laboratory by Bainbridge (1953). In addition, the need for zooplankton to migrate into patches of phytoplankton has been corroborated by field data on plankton assemblages (Dagg 1977, Mackas *et al.* 1985, Price 1989, Leibold 1990)

Although much work has been undertaken on the occurrence of barnacle larvae in relation to the physical and biological environment, only a few researchers have studied swimming behaviour of individual larvae (Hardy & Bainbridge 1954, Crisp 1955, Lang *et al.* 1980, Yule 1982, Walker & Lester 2000, Walker 2004) and information on speeds of larval stages of Cirripedia is very limited. It is clear that a combination of light, salinity, pressure, temperature, turbulence, current, and food abundance are the main factors that, in the natural environment, influence the limb beat rate and swimming speed of larvae (*op. cit.*).

Barnacle nauplii use the antenna as a main propulsive limb while the antennules and mandibles help to stabilise and to resume limb position in the recovery stroke, after which the antenna propels the body forward once again. This swimming behaviour, characterised by a series of jerks, is very different in the cyprid stage, where the body is enclosed inside a more streamlined bivalve carapace and the swimming appendages, thoracic rather than cephalic limbs, are positioned posteriorly in relation to the carapace. The metachronal rhythm of 6 rather than 2 pairs of major swimming appendages provides the cyprid with both lift as well as forward motion in a more continuous and faster movement than the nauplii are capable of (Yule 1982, Walker *et al.* 1987).

Barnacle nauplii and other zooplankton are known to alter swimming motions with feeding (Singarajah *et al.* 1967, Yule 1982, van Duren 2000), and responses to

starvation in meroplankton, which obviously decreases larval survival (Lang *et al.* 1980, Anger *et al.* 1981, Chícharo 1998, Desai *et al.* 2002), often involve altered swimming activity as a strategy to offset lack of energy input.

Swimming behaviour, speed, direction, cessation etc. can only be fully understood by combining intensive continuous sampling in the field with laboratory observation of behavioural changes in relation to specific stimuli. For all larvae, including the thoracicans, variations in swimming speed should be considered throughout larvae development. The present study assesses swimming speeds of all stages of *E. modestus* towards a light source, as well as exploring the effects of starvation on mobility of NII larvae and of two different diets on NVI. Preliminary, swimming speeds were measured also on the metanauplii of *P. pollicipes.*

V.2. METHODOLOGY

V-2.1 Preparation of larvae

Pollicipes pollicipes and *Elminius modestus* larvae were reared at a density of 1-3 nauplii mL^{-1} in a constant temperature (17.5°C ± 0.5° C) water bath in gently aerated *Rhinomonas reticulata* cultures at a density of 100 cells μL^{-1}. Following the high mortality between stages NV and NVI, part of NV larvae were fed on a mixed algal diet containing identical proportions of *R. reticulata*, *S. costatum*, *P. tricornutum* and *T. chui* to a total density of 100 cells μL^{-1}.

NII larvae of *E. modestus* were separated into two different batches. One batch was transferred to a 500 mL beaker of *R. reticulata* culture (100 cells μL^{-1}) and the other to a similar beaker containing UV filtered seawater only. Both batches were left with gentle aeration for 28 hours in a water bath at 17.5°C.

V-2.2 Recording and measurement of speed behaviour

Pollicipes pollicipes

Actively swimming NIV, NV, and NVI larvae were transferred into a 18 mL cylindrical glass dish with a flat base (height = 8 cm, ∅ = 5.8 cm). The dish contained *R. reticulata* culture at a density of 100 cells μL^{-1} and maintained at room temperature (~20.5°C). Batches of larvae taken from stock culture were allowed to stand for 1 h to arrive at room temperature prior to observation. A cold light source (Schott KL1500 electronic) provided a light source at either side of the dish. Larvae were alternately attracted to one side of the dish and then the other by alternately covering one of the light sources. The time taken for the larvae to cross the dish was measured with a digital stopwatch to a resolution of 0.01 s.

Initially, NIV swimming speeds were measured on individual larvae approximately every 3 minutes (4-8 trials) in order to assess if speeds changed over time. Priory, larvae were left to aclimate to the chamber for at least 5 minutes. Observations were then made only once on individual *P. pollicipes* NIV, NV and NVI larvae. The measured distance across the dish was 5.1 mm and the larvae were observed under a dissecting microscope (WILD M37).

Elminius modestus

Swimming behaviour of *E. modestus* larvae was recorded using a VCR (Panasonic AG 6720) and video camera (Panasonic F15 HS) attached to a dissecting microscope (WILD M37).

A 1 mm^2 grid was placed underneath the glass dish to provide distance measurements in all horizontal directions (Figure V-20). Departures from the vertical plane could be noted since the depth of field of the image was maximum 5 mm, but no accurate measurements were taken in the vertical direction. Batches of larvae taken from stock cultures were again allowed 1 h to come to room temperature (~20.5°C) prior to observation. Video recording of larval behaviour was investigated as the larvae were alternately attracted from one side of the dish to the other as described above. The basal graticule was dimly illuminated for clarity on the recorded images but at a much lower intensity than the side light sources hence did not interfere with the progress of

the larvae towards the attraction point. Speeds and directions were determined from real-time play back of the video images, using a digital stopwatch to a resolution of 0.01 s. The differences between contemporaneous larval stages were readily discernible on the video images allowing for correct stage identification.

Swimming behaviour of every larval stage was noted and detailed limb movement was recorded under 40x magnitude optical microscope.

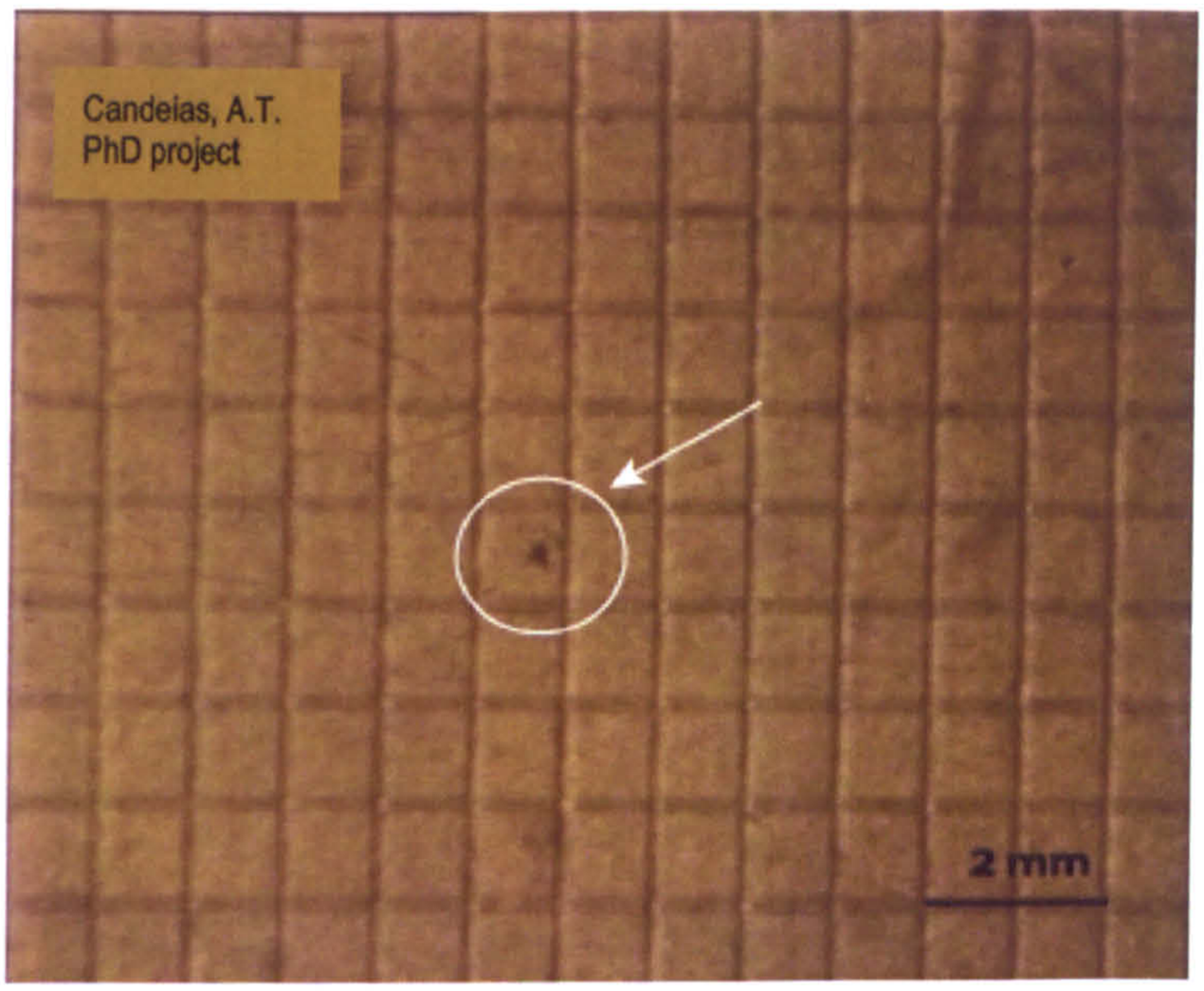


Figure V-20. Recorded image using VCR and video camera attached to a dissecting microscope, showing the 1mm^2 grid and swimming *E. modestus* nauplius I. (Low definition picture)

V.3. RESULTS

V-3.1 *Pollicipes pollicipes* larvae swimming speeds (nauplii IV, V, and VI)

Swimming speed of individual *P. pollicipes* NIV larvae steadily decreased with the number of times the animal was attracted to the light source (Pearson's correlation $r = -0.780$, $df = 28$, $p < 0.001$*) (Figure V-21). Ranging from 1.1 to 1.8 mm sec^{-1} for the first trial, speeds had dropped significantly to around 0.4 - 0.8 mm sec^{-1} by the fifth and succeeding trials. Given the potential habituation to the repeated stimulus, all further observations were carried out for one reversal of light source position only.

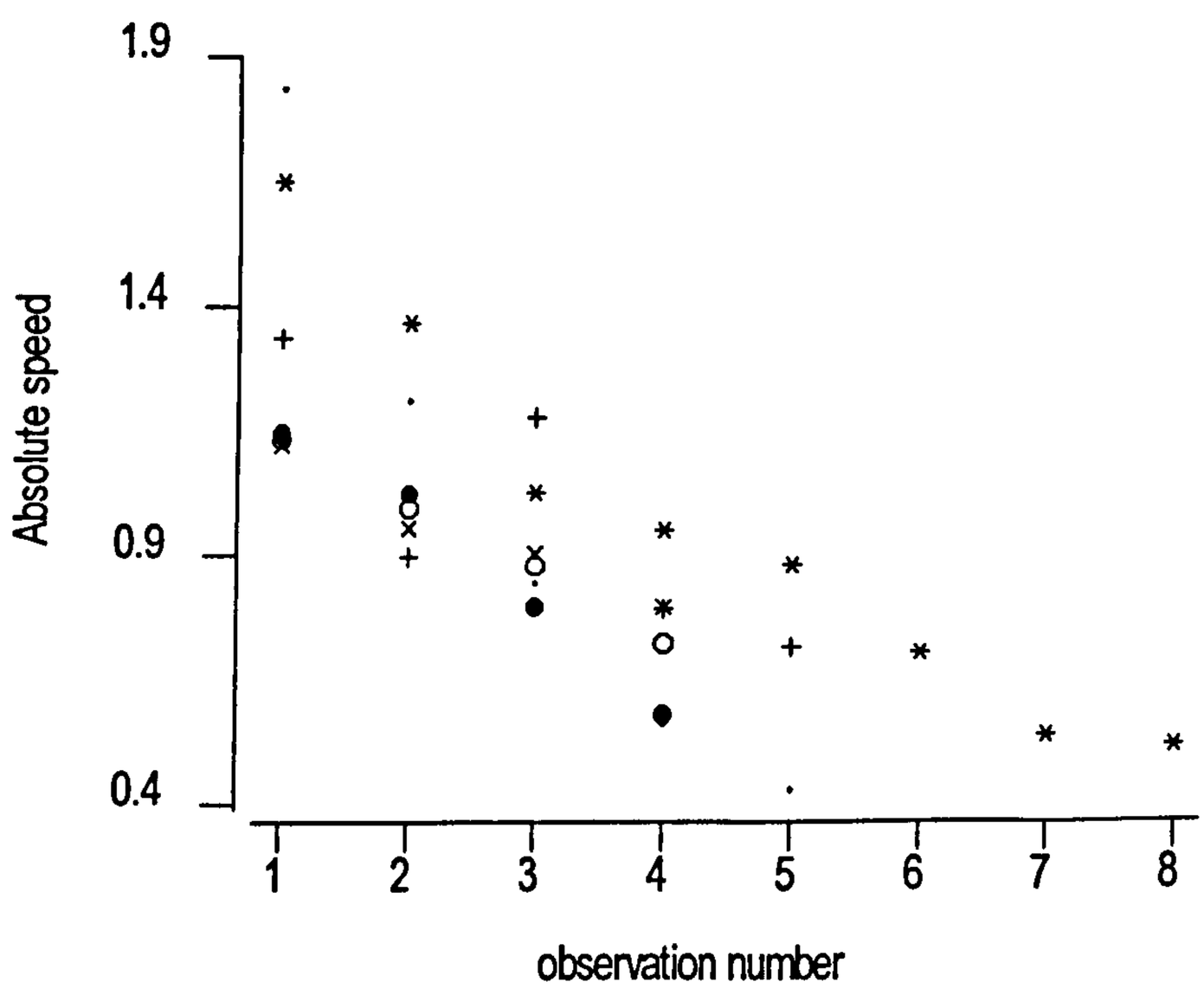


Figure V-21. Swimming speed (mm sec^{-1}) of six (different symbols) *P. pollicipes* nauplii IV measured up to eight times at approximately 3 minute intervals.

Absolute and relative speeds of *P. pollicipes* larvae increased significantly with stage (Pearson's correlation r = 0.515, df = 26, p = 0.005 and r = 0.426, df = 26, p = 0.024, respectively). Figure V-22 shows this increase of absolute speed against larval total length (see Chapter III.3.1), however, the variability also increased considerably with total length (cv ranged from 21 to 53%).

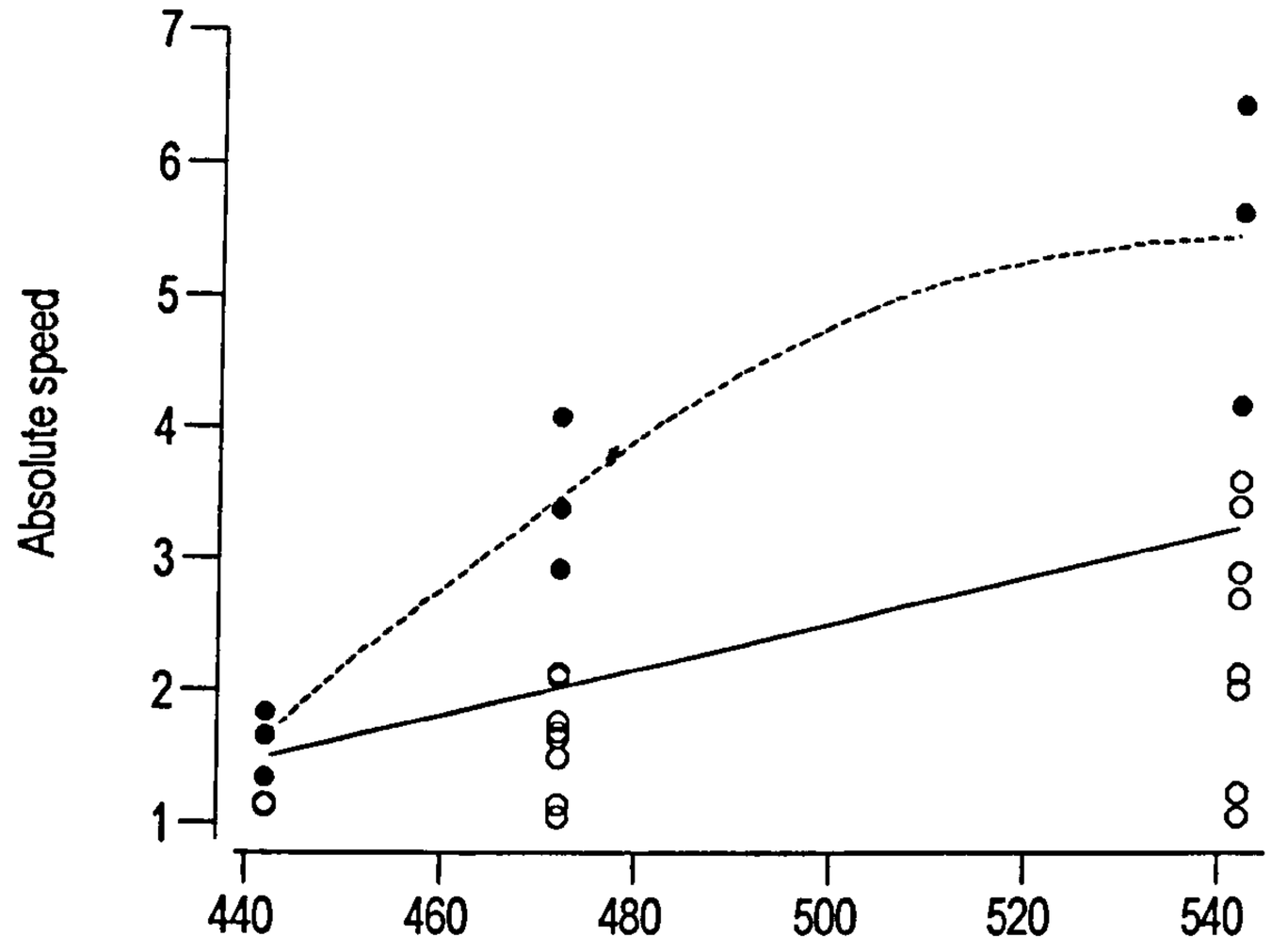


Figure V-22. The increase in swimming speed (mm sec^{-1}) of *P. pollicipes* larvae (circles) in relation to total body length (µm). Line fitted by weighted least squares regression for all the observations (continuous line). Upper curve (short dash) fitted to the 3 fasted speeds for each stage (closed circles).

The linear function fitted in Figure V-22 was weighted by the reciprocal of the variance for each stage. It indicates that, on average, *P. pollicipes* swimming speeds in *R. reticulata* cultures increase by $20.5 + 4.8$ µm s^{-1} body length $(\mu m)^{-1}$, thereby more than doubling between NIV and NVI. It is clear from Figure V-22 that there is considerable overlap in measured speeds between all 3 stages. Several sources of variability have not been taken into account such as gut fullness or moult stage hence lower than maximal speeds depended vastly on the condition of the individual larva. The 3 fastest speeds, the outer edge of the results highlighted by closed circles in Figure V-22, do not fit a linear relationship (overall Lack of Fit test p = 0.029, df = 8).

However, they do fit a cubic function:

$$\text{Speed} = -37 + 430\ \text{Length}^2 - 529\ \text{Length}^3\ \text{mm sec}^{-1},$$

$$(F_{2,6} = 18.92,\ p = 0.003,\ r = 0.863),$$

indicating that maximum swimming speeds (mm sec^{-1}) in the metanauplii are related inversely to the square and directly to the cube of the total length hence potentially the surface area and volume of the larvae. The collolary of that finding is that relative speed, expressed as body lengths sec^{-1}, should increase as a quadratic function of body length. Figure V-23 shows, again for the 3 fastest speeds measured, that relative speed does increase with body length and in inverse relation to length squared (surface area). Thus, relative speed is expressed by the equation

$$\text{Speed} = -229 + 905\ \text{Length} - 855\ \text{Length}^2\ \text{bodylengths}\ (\mu m)\ s^{-1}$$

$$(F_{2,6} = 14.44,\ p = 0.005,\ r^2 = 0.828).$$

The lower speed measurements (open circles, Figure V-23) show very little change in relative speed with increased body length as it would be expected from the linear fit obtained for absolute speed (See Figure V-22).

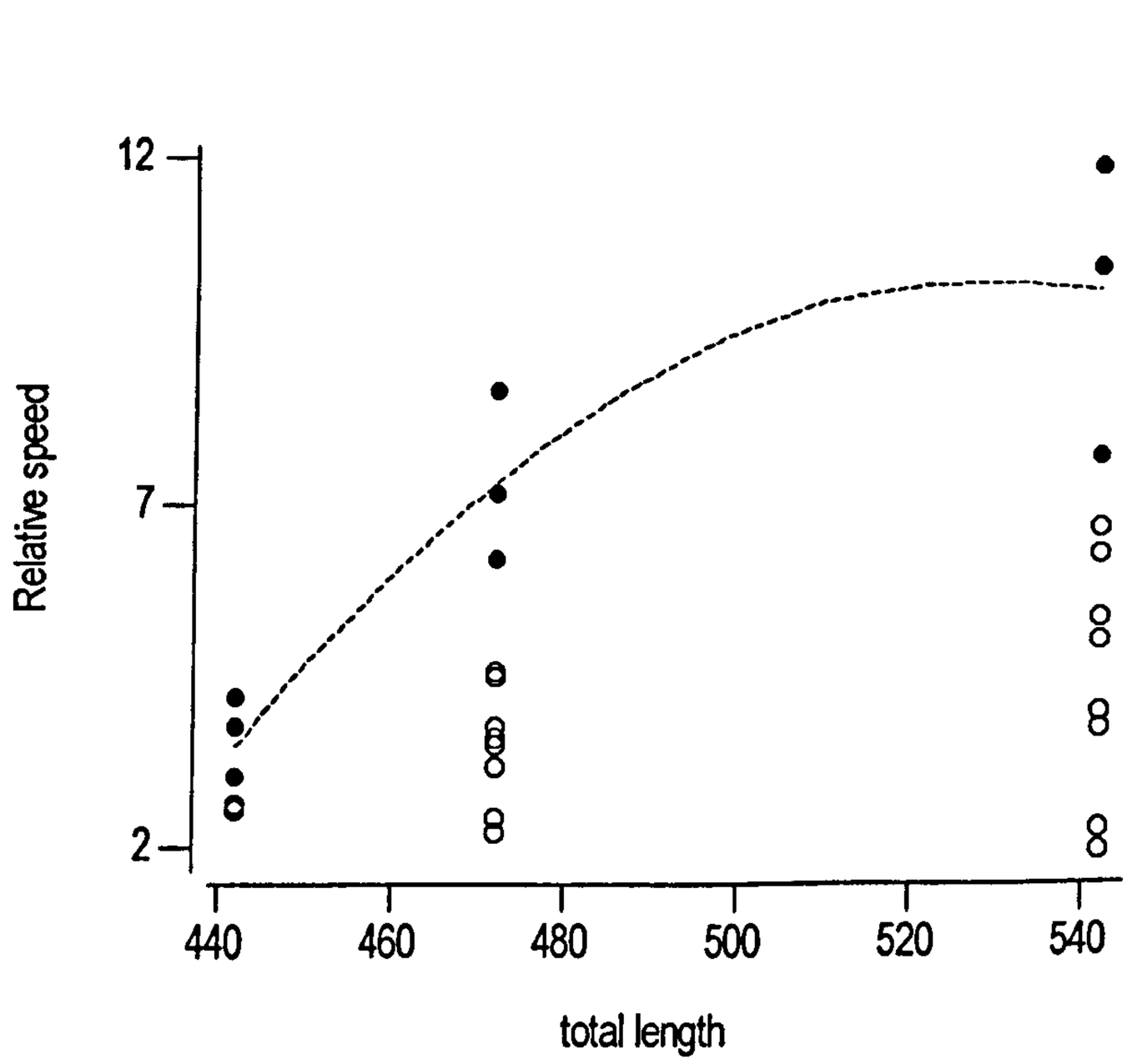


Figure V-23. Relative speed (body lengths sec^{-1}) of *P. pollicipes* nauplii IV to VI. The curve represents a quadratic function fitted to the 3 fastest speeds (closed circles) measured at each stage.

V-3.2 *Elminius modestus* larval swimming rates (nauplii and cyprid)

Naupliar and cyprid speeds

Mean (± StDev) of absolute (mm sec^{-1}) and relative speeds (body lengths sec^{-1}) of all development stages of *E. modestus* are summarised in Table V-20. Slowest absolute and relative speeds were observed for NI (1.61 mm sec^{-1} and 4.37 body lengths sec^{-1}, respectively) and the highest averages for NV (5.80 mm sec^{-1} and 11.36 body lengths sec^{-1}, respectively). Swimming speeds increased through the developmental stages but declined noticeably at stage VI.

Table V-20. The mean and standard deviation (*in brackets*) values of the absolute (mm sec^{-1}) and relative (body lengths sec^{-1}) speeds of all naupliar stages of *E. modestus* larvae.

	NI	NII	NIII	NIV	NV	NVI	Cyprids
N	83	94	75	53	86	49	19
Absolute speed (mm sec^{-1})	1.61 *(± 0.61)*	2.40 *(± 1.12)*	4.27 *(± 1.49)*	4.98 *(± 1.76)*	5.80 *(± 1.99)*	5.15 *(± 1.77)*	10.77 *(±13.82)*
Relative speed (body lengths sec^{-1})	4.37 *(± 1.66)*	6.04 *(± 2.84)*	9.81 *(± 3.43)*	10.57 *(± 3.74)*	11.36 *(± 3.90)*	9.03 *(± 3.10)*	20.22 *(±25.85)*

Figure V-24 shows all Naupliar swimming speeds are plotted relatively to total body length. (Although according to Knight-Jones and Waugh (1949), the carapace width is the most reliable estimate of nauplii size, in the current work variability of width measurements was higher than that of total length, therefore the latter was used). The speeds increase with nauplius size but exhibit the same type of variation as seen with the *P.policipes* metanauplii (see Figure V-22). NVI swimming speeds clearly do not fit the general trend being roughly 11% and 20% (absolute and relative speeds, respectively) lower than that for NV, on average (see Table V-20). The dotted line in Figure V-24 reflects a weighted (by the reciprocal of the variance) least squares regression for all stages except NVI.

The linear fit is reasonable but a degree of curvature is evident (overall Lack of Fit test $p = 0.002$) even within the wide spread of the data. In *E. modestus*, as for *P. pollicipes,* the outer edge of the distribution (here characterised by the 6 fastest speeds measured for each larval stage) was analysed separately.

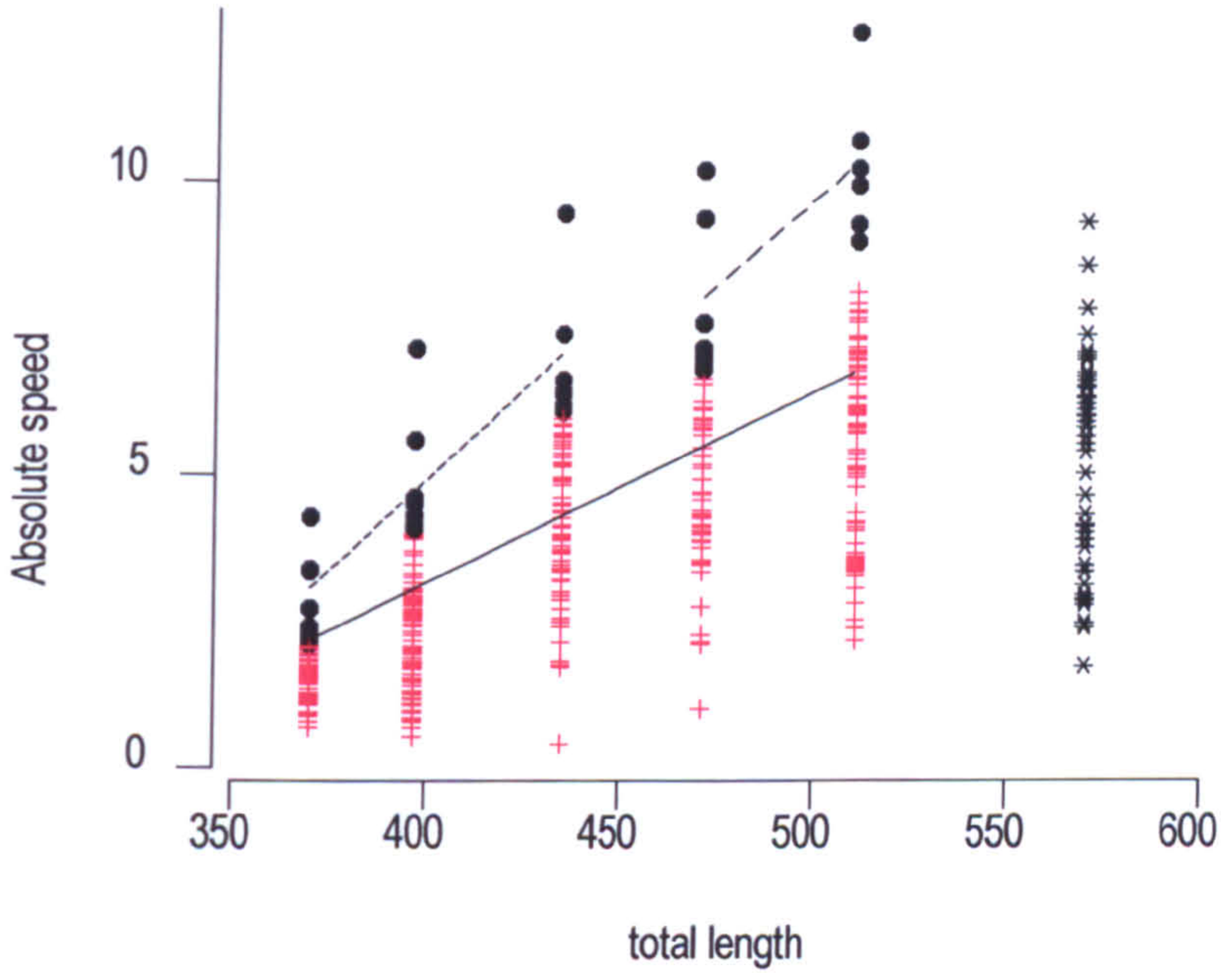


Figure V-24. Swimming speeds (mm sec^{-1}) of *E. modestus* nauplii against average total body length (μm). Lines fitted by weighted least squares regression to all observations (solid line) and to the 6 fastest measured rates of nauplii (NI-NIII, short dash line) and metanauplii (NIV-NV, long dash line). Nauplii VI (asterisks) clearly did not fit any of the trends.

The relationship between total length and swimming speed was distinctly biphasic (closed circles, solid lines, Figure V-24) with no single function giving any reasonable fit. Ultimately, two linear relationships between speed and width, one for nauplii (NI-NIII) and one for metanauplii (NIV-NV) fitted adequately. The slopes of the two relationships did not differ significantly

($Speed_{nauplii} = 61 \pm 9$ mm sec^{-1} $Length(mm)^{-1}$, and

$Speed_{metanauplii} = 56 \pm 19$ mm sec^{-1} $Length\ (mm)^{-1}$, $F_{1,26} = 0.06$, $p = 0.807$) whereas the intercept for nauplii ($-19.5 + 9.2$ mm sec^{-1}) was significantly lower than that for metanauplii ($-18.5 + 9.2$ mm sec^{-1}, $F_{1,26} = 0.01$, $p = 0.920$). The intercepts, of course, represent the impossible situation of the swimming speed of a nauplius of zero length but the difference indicates a significant change in the relationship between swimming

speed and size between larval stages NIII and NIV. The slopes of the relationships are equivalent (within the variability of the data, and being linear would suggest that there should be no significant change in relative speed with size from NI – NIII and again from NIV to NV. Table V-20 would suggest that such is probably true, on average for NIV – NV but there is a near doubling of relative speed from NI to NIII. The variability, however, is very high ranging from 35 to 47%.

Swimming speeds for a number of cyprids were also obtained and their average speed was nearly twice that of any other larval stage but the results were very variable (Range 1.76 - 64.03 mm sec^{-1}, see Table V-20 and Discussion). *E. modestus* cyprids have an average body length of 0.534 mm so the highest speed measurement equates to a prodigious 120 body lengths sec^{-1}.

Response to food type (NVI larvae)

A comparison between swimming speeds of *E. modestus* NVI larvae fed on either a mixed algal suspension or a monoculture of *R. reticulata* is shown in Table V-21. The median speed for larvae fed a mixed diet was approximately 10% lower than that for larvae fed on the monoculture but the 95% confidence intervals overlapped completely.

Table V-21. Absolute speed (mm sec^{-1}) of *E. modestus* nauplii VI under mixed algae and monoculture (*R. reticulata*) diets.

diet	N	mean (mm sec^{-1})	StDev	Median	Lower Median $CI_{95\%}$	Upper Median $CI_{95\%}$
Mixed	23	4.86	2.72	3.89	3.34	6.20
R. reticulata	49	5.15	1.77	5.39	4.59	5.66

The speeds for the larvae fed *R. reticulata* were approximately normal (Anderson-Darling $A^2 = 0.544$, $p = 0.154$) but those for larvae fed the mixed diet were not ($A^2 = 1.167$, $p = 0.004$). Since the shapes of the distributions were roughly similar, medians were compared by Mann-Whitney U-test and the difference was shown not to be significant ($W = 738$, $p = 0.223$).

Response to starvation (NII larvae)

Figure V-25 shows the wide spread of the speeds obtained for fed and unfed *E. modestus* NII larvae over 28 hours. There was no correlation between swimming speed and time for either the unfed larvae (Pearson correlation $r = -0.03$, $df = 267$, $p = 0.625$) or the fed larvae ($r = 0.199$, $df = 87$, $p = 0.062$).

Table V-22 summarises the swimming speeds of the larvae in the two conditions. There was a 16% reduction in average swimming when the larvae were deprived of food. The variability was, however, high (cv = 37% and 42% for unfed and fed larvae respectively).

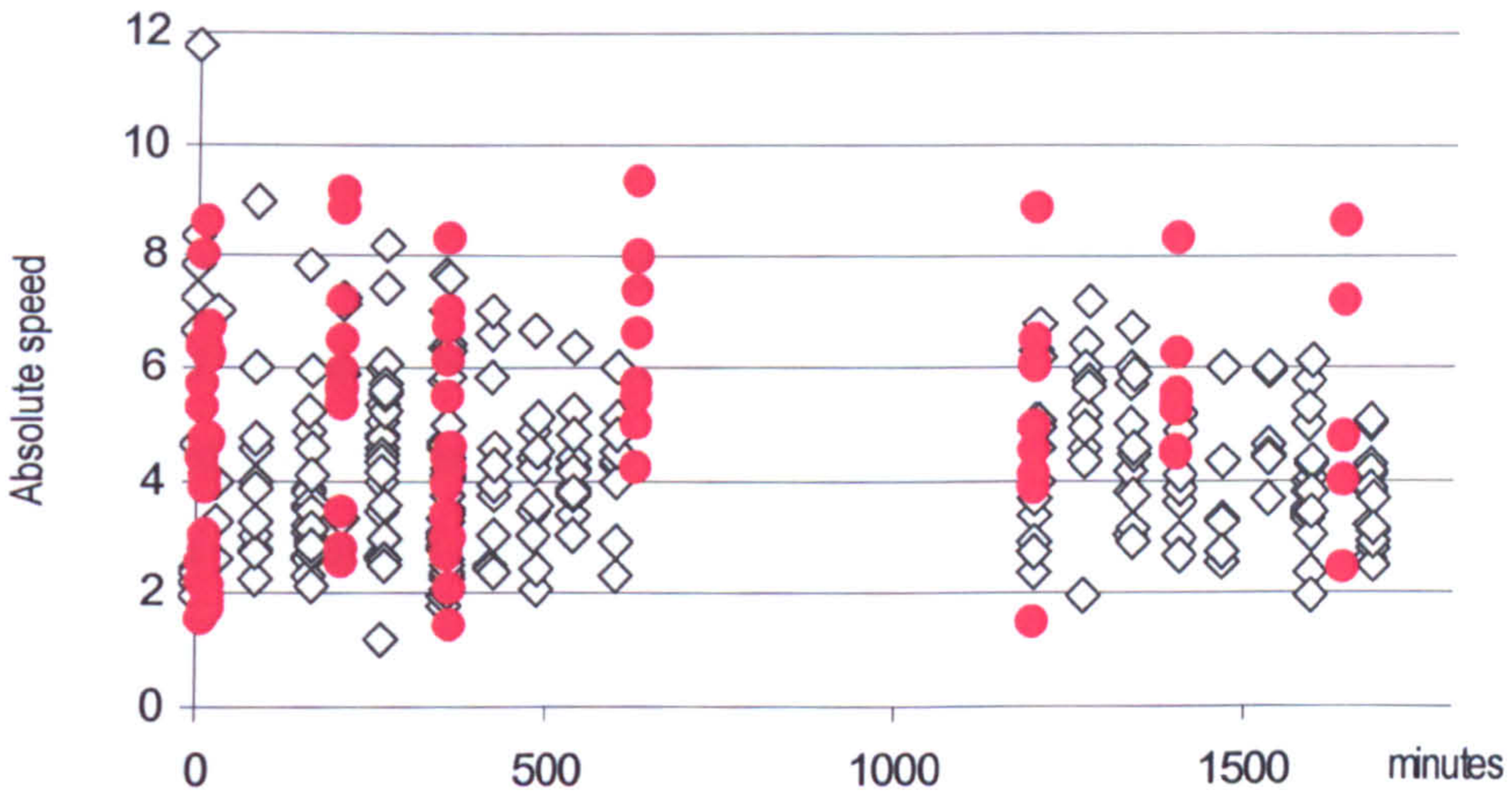


Figure V-25. Swimming speed (mm sec^{-1}) of *E. modestus* nauplii II under feeding (*R. reticulata*, 100 cells μL^{-1}, red circles) and starving (UV filtered seawater, diamonds) regimes.

Table V-22. Swimming speeds (mm s^{-1}) of *E. modestus* larvae (NII) either deprived of food (Unfed) or fed a monoculture of *R. reticulata* over a 28 hour period.

Diet	N	Mean (mm sec^{-1})	Stdev	Median	Lower Median CI 95%	Upper Median CI 95%
Unfed	269	4.18	1.54	3.97	3.88	4.26
Fed	89	4.95	2.07	4.77	4.44	5.40

The speeds for fed larvae were approximately normally distributed (Anderson-Darling $A^2 = 0.47$, p-value $= 0.241$) but those for unfed larvae were decidedly non-normal ($A^2 = 3.045$, $p < 0.001$). The shapes of the distributions were, however, broadly similar (discounting a small number of high speed outliers for the unfed larvae) hence the difference between medians was tested by Mann-Whitney U-test. The median speed for larvae deprived of food proved significantly lower ($W = 45558$, $p = 0.013$) than that for larvae fed on *R. reticulata* by some 0.3-1.3 mm sec^{-1} (CI 95% of the difference). Median swimming speeds for feeding larvae were thus some 20% higher than those of non-feeding larvae.

V-3.3 Swimming behaviour description of *Elminius modestus* larvae

The swimming behaviour of *E. modestus* larvae is very variable. Although the limbs appear to perform the beat in the same manner, irrespective of conditions, the speed and direction of the whole organism varied with conditions. Quantitative observations on swimming speeds, reported above, were complemented by qualitative observations on larval behaviour.

The proportion of larvae swimming towards the light appeared highest for the earlier larval stages. The cypris larvae for example were very reluctant to respond to changes in light direction.

Nauplii showed two major types of swimming behaviour. An unidirectional, fast, continuous and straight traverse to the light source was characteristic of starved larvae. A slower speed traverse, characteristic of nauplii with full guts, was marked by slower limb beats with a clear pause between the propulsive and the recovery strokes, which resulted in lower speed. Without a strong directional stimulus from a point light source, larvae would adopt a sinusoidal course or even swim in small circles, often involving "backward somersaults". The frequency of the second behaviour increased with development stage (other than the cyprids) and in well fed animals.

Swimming tended to be virtually continuous in NI & NII but after the moult to NII, swimming regularly alternated with a pause, for less than 1 second, resulting in sinking. During the pause, larvae either fully extended their limbs or bent and curled

them across the ventral thoracic process once or twice (see Yule 1982).

Larvae also responded quickly to, and appeared to sense, obstacles. When approaching other nauplii in the culture, they could rapidly change direction, go around each other and avoid collisions. Indeed collisions were rarely seen during the observations in slow playback of the recorded images at higher magnitude.

V.4. DISCUSSION

Increased larval speeds of *E. modestus* during earlier metamorphosis might be associated with particular requirements: initially, for larval dispersal and subsequently, for nutritional requirements and change in behaviour towards a settled form. These results are confirmed with measurements obtained from *P. pollicipes* metanauplii.

E. modestus are essentially small, slow moving organisms swimming through a world in which viscosity and not inertia dominates, and this can be analysed under the scope of fluid dynamics.

Calculations of Reynolds' number (Equation V-1) relative to the present data provided valuable information for the interpretation of the results:

$$\mathrm{Re} = \frac{DV\rho}{\mu} \qquad \ldots \text{Equation V-1,}$$

where D is the larval total body length (m), V the absolute speed ($m\ s^{-1}$), ρ ($Kg\ m^{-3}$) and μ ($Kg\ m^{-1}s^{-1}$) the suspension density viscosity. The kinematic fluid viscosity (η) - coefficient between ρ / μ - of sea water was considered as equal to $1.1\ s\ m^{-2}$.

The fastest nauplii (stage V) move at just over 1 $cm\ s^{-1}$ equating to a Reynolds number of 6. As the larvae develop through the 6 naupliar stages they also increase in size with larger propulsive limbs and greater musculature. Their body surface area also increases which increases the amount of resistance to propulsion they experience. The range of Reynolds numbers varied from 0.2 for the minimum speeds recorded (N1-NIII) to 5-6 for the maximum speeds of NIV-NVI larvae.

The cyprid larvae reached much higher speeds with one larvae recorded at 6.4 $cm\ s^{-1}$ equating to a Reynolds number of 30. Above Reynolds numbers of 1 inertial

effects take on increasing importance (e.g Larval fish, Sfakiotakis *et al* 1999, Ascidian larvae, McHenry & Patek 2004) and form drag (often called pressure drag) begins to play an increasing role in the resistance to motion. The nauplii of *E. modestus* swim in an environment where shape is less important in determining swimming performance than is surface area. By the time they develop towards the final nauplius stage their swimming performance seems reduced over that at the preceding NV stage (maximum recorded speed of NVI larvae was 9.3 mm s^{-1} and that of NV larvae was 12.6 mm s^{-1}). The reduction in speed may be a consequence of their increased size bringing them towards the point where shape is becoming more important in determining resistance to movement (both for the limbs and the body as a whole) making increased speed impossible despite increased musculature.

Two major differences occur at the metamorphosis from NVI to cyprid (a) the broad flat carapace folds in half to enclose the cephalic appendages and adopt the smooth, streamlined cyprid appearance (b) six pairs of posteriorly positioned thoracic appendages propel the organism. These changes result in a more compact frontal area projection, a prerequisite for reducing form drag, and a concentrated, metachronal engine producing thrust along the mid-line of the body rather than at either side (see Yule 1982). The metamorphosis to the cyprid stage seems a prerequisite for settlement in all barnacles and occurs in similar fashion in all. Although swimming speeds of *P. pollicipes* cyprids were not obtained during the current study, the parallels of decreasing swimming ability in the later, metanaupliar stages were still seen, if not as dramatically at NVI. Maximum swimming speeds in *P. pollicipes* ranged from 2 mm s^{-1} in NIV to 6.5 mm s^{-1} at NVI (equating to Reynolds numbers from 0.8 to 3.3). Thus, considering their generally larger size than *E. modestus* larvae, the transition from metanauplius to cyprid is occurring when size and potential speed equate to similar types of flow and increasing importance of form drag.

Starvation in the early stage of the life-cycle of barnacle nauplii has the effect of prolonging larval development and decreasing survival, either directly or indirectly by increasing the chance of mortality due to predation or dispersal of larvae to unfavourable conditions. Lang & Marcy (1982) indicated that decrease of *Balanus improvisus* survival in the subsequent stages is irreversible after 24 hours of starvation from hatching. The observations made in the current study on food deprivation compared fed larvae swimming in an algal culture and unfed larvae swimming in clean filtered seawater. At least two factors impinge on the outcome; (a) the effects of algae

on swimming speeds and (b) the effects of food deprivation on swimming speeds. Over the 28h period investigated, no effect of food deprivation on nauplius swimming speeds was detected (no correlation between speed and time). Presumably, a rapid photopositive response is always elicited from starved NII *E. modestus* as long as the larvae have the stored energy to do so. The imperative would be to find food algae and, as has been proposed many times, photopositivity should bring larvae to the upper reaches of the water column where the probability of encountering food algae should increase. There is no evidence from the current results that *E. modestus* larvae were suffering depletion of energy reserves during the 28 hours of starvation. Indeed, it has been reported that NII barnacle larvae carry enough yolk from the egg to allow a moult to NIII without the need for food (Yule, *pers. comm.*). The degree of starvation may well impinge on later survival but at NII it would appear that reserves allow the larvae at least 28 hours to find a food source.

Swimming speeds for larvae that, in all probability, could not have been suffering energy depletion (i.e. fed continuously) also did not alter significantly with time over a 28 h period. When measured in the presence of food algae, the swimming speed, during their photopositive response, was 20% greater than that of starved larvae swimming in clean seawater. It has been suggested that planktonic herbivores can increase the rate at which they handle water in the presence of food algae (*Temora longicornis*, Yule & Crisp 1983; *E. modestus* and *S. balanoides* nauplii, Yule 1982, Walker *et al.* 1987). For the way in which *E. modestus* nauplii feed it might well be that the response to food availability, a 10 – 50% increase in the volume swept by the antennae, depending on algal concentration (Yule 1982) results in a 20% increase in swimming speed in the current context.

Although through laboratory experimentation, the mixed algal diet produced better survival hence more *E. modestus* stage VI nauplii and cyprids than did the *R. reticulata* culture, no significant swimming speed differences were found between the respective larvae. The mixed diet produced higher survival rates but those larvae which survive on the 'less suitable' diet show no detriment to their swimming capabilities. Since the larvae were not exposed to a different diet from the one they were reared on during the trials it is not possible to state whether the presence of different algal species in the water affects the swimming of these nauplii.

The lack of published work on Cirripedia larval speeds limits comparisons between species. Walker (2004) showed that swimming speeds of the lecithotrophic

Heterosaccus lunatus decreased with naupliar development. These larvae have no requirement to find food but do need to conserve energy; their dispersal is a balance between distance and energy consumption. Comparatively, planktotrophic larvae need to feed and in feeding increase in weight throughout their larval existence. Increased swimming speed is presumably a necessity to continue food gathering as the larvae increase in weight. Lecithotrophic larvae lose weight during their development as they lose body musculature and consecuently swimming energy.

Both planktotrophic and lecithotrophic barnacle species have lecithotrophic settlement stage cyprids with radically different shapes to the nauplii. Table V-23 compares cyprid swimming speeds of several barnacle species.

Table V-23. The mean values of absolute (mm sec^{-1}) and relative (body lengths sec^{-1}) speeds of cirripede cyprids (range in brackets). See text.

Species	Carapace length (µm)	Absolute speed (mm sec^{-1})	Relative speed (body lengths sec^{-1})	Reference
Balanus crenatus	900	39.0	43.3	Crisp, 1955
Semibalanus balanoides	1000	48.0	48.0 (41 - 60)	Crisp, 1955
Semibalanus balanoides	1000	53.46	53.46 (15 - 94.8)	Yule, 1982
Heterosaccus lunatus	(m) 305 (f) 202	25 - 33.3 15.9 - 21.7	91.6 (81.9 - 109.2) 87.1 (78.6 - 107.6)	Walker & Lester, 2000
Heterosaccus lunatus	(m) 288-304	9.9 (7.6 -12.1)	33.7 (25.1 - 40.4)	Walker, 2004
Sacculina carcini	(m) 270 (f)230	17.7 13.5	65.6 58.7	Werner, pers. comm. *in* Walker, 2004
Elminius modestus	534	10.77 (1.76 - 64.03)	20.18 (3.29 - 119.91)	present study

(f) female, (m) male

Highly variable velocity is an intrinsic characteristic of the cyprid stage, which results from highly variable swimming behaviour. The fastest motion reflects the efficiency of the fusiform shape and the thoracopod propulsion while the slower swimming speeds result from 'exploratory' behaviour, where cyprids scrutinise the substrata with their antennules. Maximum measurements on *E. modestus* are slightly higher (120 body lengths sec^{-1}) when compared with other thoracicans. In *Semibalanus balanoides*, Crisp (1955) reported a maximal speed of 60 body lengths sec^{-1} whilst Yule (1982) measured a maximum of 95 body lengths sec^{-1}. On the other hand, the mean values of all other species reviewed in Table V-23 were greater than that of *E. modestus*, which departed immensely from the highest values due to the slowest speeds registered on this species. Speeds higher than 64 mm sec^{-1} could not be recorded thus the fastest individuals were not equally sampled as those with exploratory behaviour.

Crisp (1955) concluded that cyprids activily swim resisting the forces of currents up to velocity gradients of 500 sec^{-1} and that the ability to attach under conditions of water flow influence animal distribution and survival. Accordingly, the maximal cyprid swimming speeds shown by all barnacle species is the successful adaptation to resist viscosity and shape drag, particularly high during this settling period.

Video recording has proved to be very useful for measurements and observations of larval behaviour. Nevertheless, processing video images is time consuming and laborious work. Computerised image analysis is now becoming more common as the technology becomes more available and affordable. Van Duren (2000) and Gree *et al.* (2003) have analysed the response of copepods to the presence of food, predators and mates using the vectoral analysis of distance combined with time, to assess swimming modes and speeds. The application of these techniques to barnacle larvae would help to identify changes that are too quick to be detected by conventional video recording.

VI Respiration rates and metabolism of *Elminius modestus* larvae

The respiration of *Elminius modestus* nauplii was measured under starvation and different algal diets, using an accurate polarographic method. Immediately after hatching, oxygen consumption was 4 nlO_2 $animal^{-1}$ $hour^{-1}$, decreasing to a stable rate of approximately 0.5 nlO_2 $animal^{-1}$ $hour^{-1}$ after 8 hrs. As a result, the weight specific routine respiration rate of unfed nauplius II was 0.54 nlO_2 $animal^{-1}$ $hour^{-1}$, comparable to lecithotrophic species larval rates. Throughout development, oxygen consumption of larvae feeding on *Rhinomonas reticulata* increased until nauplius V, decreasing during the last feeding stage. During earlier stages (nauplii I to IV), metabolic activity was higher on larvae fed on *Pavlova lutheri* and *R. reticulata*, while rates on *Skeletonema costatum* and *Tetraselmis chui* indicated low nutritional value of these diets. General higher respiration rates on algal mixture diets suggested a considerable extraction of nutrients as well as higher energy expenditure on digestive metabolites. Average respiration rates of feeding nauplii were 2.2 times higher than non-feeding ones. Differences in relative respiration rates between nauplii and metanauplii denote dramatic physiological changes during this moult.

VI.1. Introduction

Understanding the ecology of barnacle larvae requires a comprehensive knowledge about the biology of this group of planktonic crustaceans. Measurement of oxygen consumption provides information on their metabolism and under the scope of physiological energetics, analysis of respiration has been undertaken on larvae of decapods (*Carcinus maenas*, Dawirs 1983; *Libinia ferreirae*, Anger *et al.* 1989; *Penaeus monodon*, Kurmaly *et al.* 1989a) and bivalves (*Mytilus edulis*, Sprung 1984a, b; *Ostrea edulis*, Crisp *et al.* 1985, Beiras & Pérez Camacho 1994), embryos of fish and larvae (*Gadus morhua*, Finn *et al.* 1995; Anguilliformes, Bishop & Torres 2001), larval krill (*Euphrasia superba*, Frazer *et al.* 2002), cladocerans (*Daphnia parvula*, Porter & McDonough 1984; *D. magna*, Pirow *et al.* 1999) and copepods (Pavlova 1994; *Temora longicornis* Castellani 2001; *Acartia torsa*, Thor 2003).

Temperature (Verity 1985), body mass (Prosser & Brown 1962), type and quality of food (Champalbert & Pagano 2002, Frangoulis *et al.* 2005), container volume and experiment duration (Wen & Peters 1994), light (Porter 2001) and pollutants (St-Amand *et al.* 1999) have an effect on respiration rates of zooplankters. Oxygen

stress (by absence) is known to control zooplankton survival and recruitment (Roman *et al.* 1993) and barnacles might show some level of physiological adaptation to low-oxygen waters (Achituv e*t al.* 1980) though the extent of this stress resistance is yet to be investigated.

Oxygen consumption of barnacle larvae has received modest attention during the last half century. Significant works were presented by Crisp (1974b), Lucas (1980), Lucas *et al.* (1979), and Harms (1987), with the latter being the only study fully dedicated to all the larval stages of a planktotrophic thoracican species, *Elminius modestus.* Combining respiration rate with biomass and food content analysis, Harms (1987) constructed a model for the energy budget of *E. modestus* larvae, discussing the pre-adaptations for the successful immigration of this species throughout Europe. Additionally, Collis (1991) and Walker & Collis (1995) analysed the larval respirometry of the parasitic *Sacculina carcina* and compared it with other lecithotrophic (and planktotrophic) larvae.

The energy required for planktonic swimming and exploration determines the food and/or reserves requirements of each larval stage and the oxygen consumption shows how much energy is being metabolised, which in turn reflects the physiological state of the organism. Nauplii have considerable impact on the phytoplankton community and research on feeding metabolism allows estimation of their participation in the energy flux within marine food webs.

The present study investigates food suitability based on respiration rates of *E. modestus* nauplii under different diets. It also analyses the effect of starvation on newly hatched larvae and discusses the immediate effects of food absence during larval development. The surface-to-volume ratio in barnacle larvae is sufficient large to permit integumentary respiration (Graham 1988 *in* Pirow *et al.* 1999), which was measured using a highly accurate polarographic method.

VI.2. METHODOLOGY

VI-2.1 Larval dry-weight

Following the protocols of Harms (1987) and Collis (1991), several methods were tested for adequacy in ascertaining the dry weight of *Elminius modestus* larvae. Whatmann glass fibre papers crumbled on drying loosing significant and variable weight during manipulation. The numbers of larvae sieved onto nylon plankton netting prior to drying were both difficult to ascertain and impossible to guarantee during manipulation for weighing. The most reproducible results were obtained by using aluminium foil boat, as also determined by Collis (1991).

Cylindrical foil boats, all weighing less than 17 mg with a diameter of 5 mm, were constructed from squares of aluminium foil pressed in a cleaned plastic tube mould. The boats were oven dried overnight at 50°C and individually weighed before transference of the larvae. Actively swimming larvae were transferred from culture, into solid watch glasses containing 3.9% isotonic ammonium formate solution (3.9% by weight). The larvae were washed 6 times with isotonic ammonium formate solution to remove all traces of salt.

300 larvae were then individually counted with the aid of a tally counter into a solid watch glass containing distilled water, twice (see Collis, 1991). Batches of washed larvae were transferred to the aluminium boats using a Pasteur pipette. The larvae were individually recounted as they were transferred to the boat. Excess fluid was removed from the boats and any larvae inadvertently withdrawn were subtracted from the remaining total. Three replicates each containing 300 larvae were prepared for each naupliar stage. The replicate samples were dried at 60°C (Lovegrove 1966 *in* Postel *et al* 2000), for at least 8 hours, surely to constant weight (i.e., no change in dry weight in re-weighing), after which they were cooled in a desiccator. They were then weighed on a Cahn model 32 Microbalance to a resolution of 1 μg.

VI-2.2 Respirometry

Larval respiration rates were measured using polarographic electrodes (Strathkelvin model 1302) connected to an Oxygen meter (Strathkelvin model 781), with the voltage output recorded by a data logger (LogIT, model SL). Each ρO_2 – electrode fitted the bottom of a respiration chamber (Strathkelvin, RC200), which included a water jacket furnished with water at constant temperature from a thermo-circulator (Grant LTD 6), at 17.5 ± 0.5°C. The water reservoir of the thermo-circulator was also used to maintain the larval cultures prior to the measurements.

Chambers were thoroughly cleaned at the beginning of the procedure, first with 1% sodium hypochlorite solution, followed by distilled water. Each ρO_2 - electrode was calibrated to 0% oxygen concentration with sodium dithionite dissolved in seawater, and 100% saturation was achieved with agitated fine-filtered, UV irradiated seawater. Larvae were pipetted into the respirometer chambers in volumes of between 150-300 µL. The chambers were then closed off using their pressure relief caps and the whole chamber covered with a sealed cardboard tube to prevent light cues aggregating the larvae.

Mechanical mixing of the respirometer seawater is not possible due to the small size of the RC200 chamber which relies on the natural movement of the occupants to keep the water stirred and the oxygen concentration homogenous. Oxygen uptake was measured over at least 30 minutes but always ensuring that oxygen concentration in the water did not fall below 80% of air saturation (Belman & Childress 1973). Blank readings (oxygen consumption in the absence of larvae) were obtained by measuring oxygen concentration changes in equivalent volumes of seawater but without larvae. Blank measurements were conducted at the start and end and occasionally in the middle of each day. Between each consumption rate measurement, the chambers were cleaned with UV filtered seawater and dried with absorbent paper. At the end of experimental observations, the larvae were carefully pipetted from the respirometers into solid watch glasses, avoiding any loss of or damage to individuals. The numbers of living larvae were counted and their stage determined under a dissecting microscope, while another run was carried out.

Data log and calculations

'Insight 2' version 2.23 and 'LogIT Lab 2 Standard' version 2.02J2 were used to record and analyse the oxygen meter voltage output. After introducing larvae into the respiratory chambers, electrode output (proportional to oxygen concentration) took approximately 5 minutes to stabilise. Oxygen consumption rates were calculated using the linear regression of the drop in oxygen concentration over time for the succeeding 10 minutes. At each run, maximum solubility of oxygen was calculated according to Green & Carrit (1967). All larval consumption rates were adjusted for the number and stage of larvae in the chamber and any change measured in the blank readings.

VI-2.3 Preparation of larvae

Elminius modestus adults were collected from the Menai Bridge pier and NI larvae released as described in Chapter II. The newly hatched nauplii were immediately transferred to containers filled with UV filtered sea water at a density of 1-3 animals mL^{-1}. During the experiment, the larvae were maintained in a temperature-controlled bath at 17.5°C (±0.5°C), under constant illumination and gentle aeration.

Newly hatched larvae

Oxygen consumption rates of stage I nauplii were measured immediately after hatching. The larvae were then left for 19 hours further under the same conditions and oxygen consumption measurements were then made with larvae from the same batch, which had already moulted to NII.

Different diets

Further batches of newly hatched NI larvae were transferred into each of six different algal suspensions to a final density of 1-3 animal mL^{-1}. Four of the diets consisted of monocultures of *R. reticulata*, *P. lutherii*, *S. costatum* and *T. chuii*, while

the fifth and the sixth were mixed cultures of *R. reticulata* + *S. costatum* and of all four algae, respectively, in equal proportions (by cell density). The total algal cell density was 100 cells μL^{-1} in all suspensions. Oxygen consumption measurements were obtained for all stages from NII to NV (NIV on larvae feeding *R. reticulata* & *S. costatum*), as described above. Due to poor larval survival on the other diets, NVI larvae were only obtained from cultures fed *R. reticulata* and the mixed algal culture.

Feeding and non-feeding larvae

Yet more batches of larvae were fed from hatching with *Rhinomonas reticulata* cultures at concentrations of 100 cell μL^{-1}, as described in Chapter II. The cultures were reared through the larval stages. As each stage was obtained, individuals of the appropriate stage were sampled from the cultures. Half these larvae were fed *Rhinomonas reticulata* at 100 cells μL^{-1} (fed) and the other half maintained without food in clean UV-filtered seawater (starved). Oxygen consumption of larvae from both treatments was measured over time from when the starved batch was deprived of food.

VI.3. RESULTS

VI-3.1 Dry weight of *Elminius modestus* nauplii

Table VI-24 shows the mean dry weight (DW) of *Elminius modestus* nauplii at each stage. From hatching as NI larvae weighing half a µg, their average weight increased more than tenfold through the naupliar stages. Figure VI-26 illustrates how dry weight increased exponentially with naupliar stage, and a significant linear relationship ($r = 0.995$, $df = 16$, $p < 0.001$) was observed between the natural log of weight and a numerical representation of stage (NI = 1 through to NVI = 6)

$$\text{Dry Weight} = 0.3.e^{0.47 \times \text{Stage}} \qquad \text{.....Equation VI-1}$$

Equation VI-1 defines the increase in dry weight (µg) with stage. The constant (estimated weight at NI) was 0.48 µg which was noticeably lower than the measured average for stage I nauplii of 0.52 ± 0.06 ($CI_{95\%}$). The difference may indicate a lack of fit for Eq. VI-1 to the data for the earlier larval stages, which appear, from Figure VI-26, to have a slower rate of relative growth than do the metanauplii (NIV - NVI). At each metamorphosis, average increase in dry weight approximately 50% (see Table VI-24) which is generally confirmed by the exponent of Equation VI-1 that suggests a 47% increase in dry weight from stage to stage.

Table VI-24. Mean dry weight, standard deviation and range for all six *E. modestus* naupliar stages (n=3).

stage	Direct weight (µg animal^{-1})		
	Mean	StDev	Range
NI	0.521	0.029	0.490 – 0.547
NII	0.751	0.054	0.703 – 0.810
NIII	1.107	0.037	1.070 – 1.143
NIV	1.898	0.092	1.810 – 1.993
NV	3.001	0.045	2.957 – 3.047
NVI	5.488	0.165	5.330 – 5.660

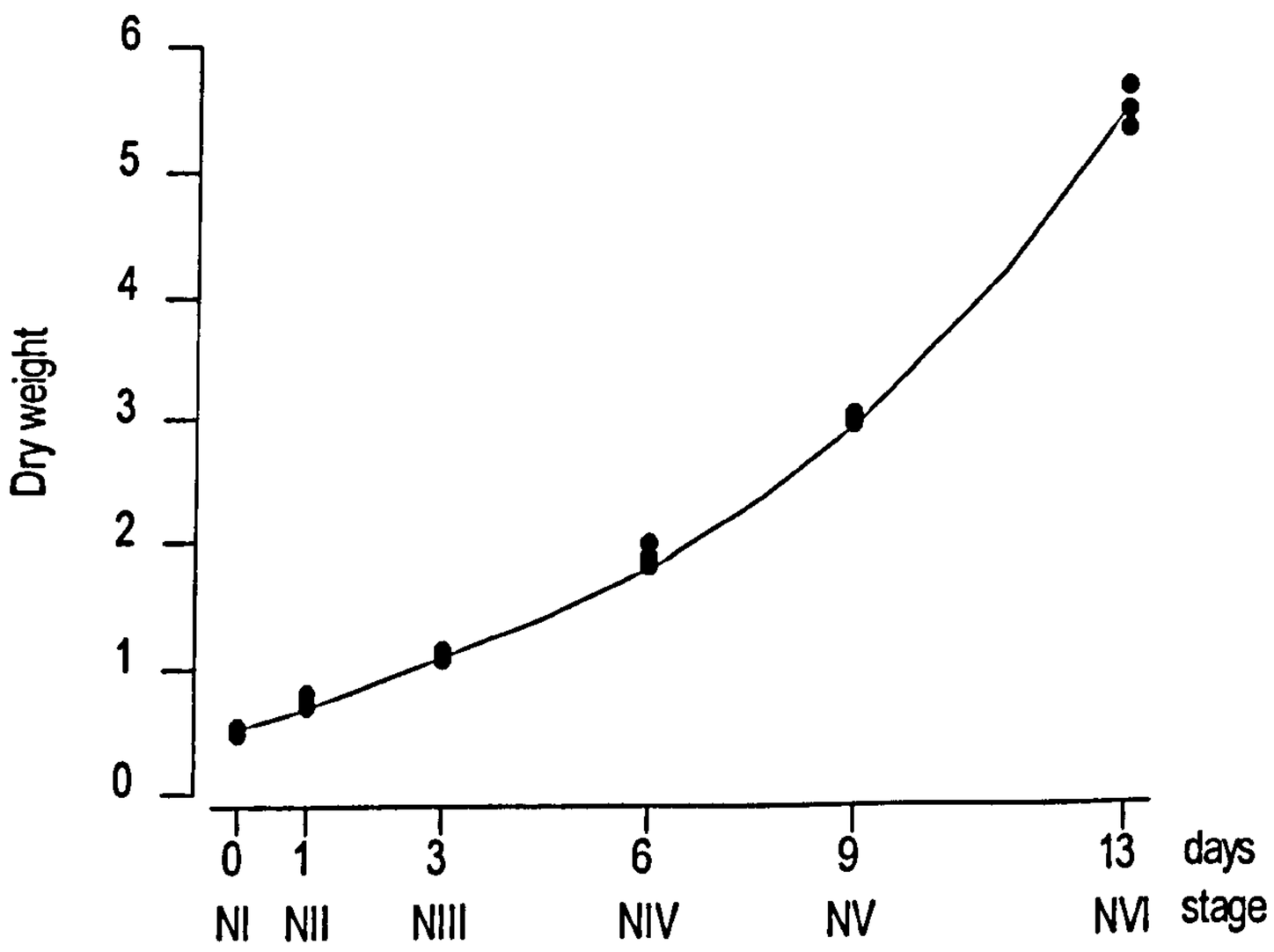


Figure VI-26. Dry weigh (µg) of *E. modestus* nauplii.

VI-3.2 Oxygen consumption of newly hatched larvae

Oxygen consumption rates of newly hatched *E. modestus* decreased significantly with time from hatching ($r = -0.789$, $df = 27$, $p < 0.001$), clearly illustrated in Figure VI-27. Immediately after hatching, oxygen consumption was around 4 nl O_2 animal^{-1} hour^{-1} decreasing to a stable rate of around 0.5 nl O_2 animal^{-1} hour^{-1} after roughly 8 hours. Over the first 8 hours of observation, the consumption rate fell by 0.46 ± 0.19 nlO_2 animal^{-1} hour^{-2} (SLR slope ± SE). Low values of respiration after 8 hours showed no significant correlation with time ($r = 0.015$, $df = 15$, $p = 0.956$) and averaged 0.54 ± 0.09 nl O_2 animal^{-1} hour^{-1}.

NI larvae metamorphosed during the experiment such that after 8 hours most of the larvae were at NII. Since stage II nauplii weigh approximately 0.7 µg, the mean weight specific routine respiration rate of unfed stage II (8~19 hours) *E. modestus* nauplii was 0.721 nlO_2 µgDW^{-1} hour^{-1}.

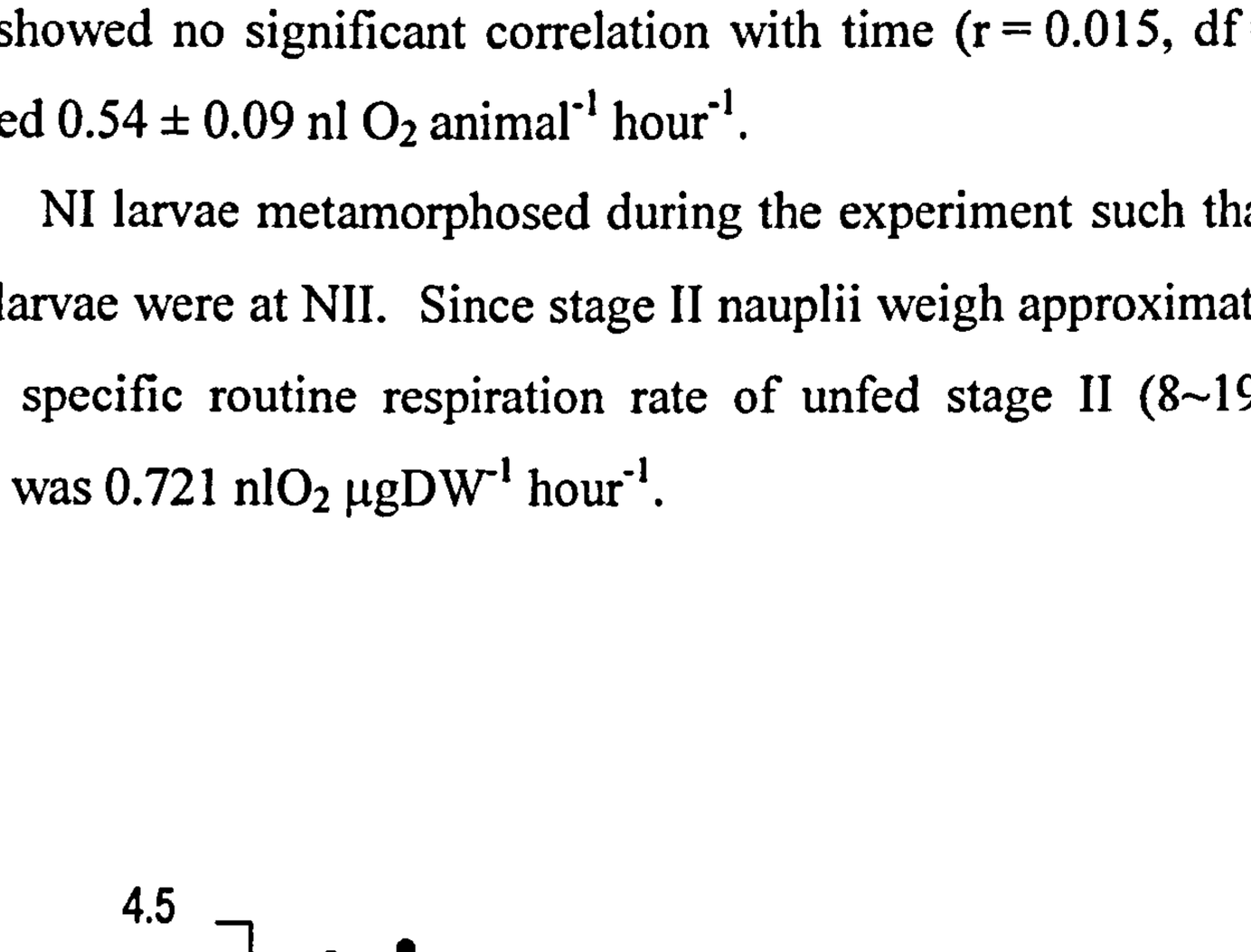


Figure VI-27. Oxygen consumption (nlO_2 animal^{-1} hour^{-1}) by unfed *E. modestus*, immediately after hatching, along time (minutes). Continuous fitted line refers to significant regression analysis and dashed-line shows the main trend after 8 hours of experiment, fitted by eye.

VI-3.3 Respiration: different diets, different stages

Due to differential larval mortality in the experimental food regimes, oxygen consumption was not measured at all stages for every diet. The *R. reticulata* and *S. costatum* mixture never produced larvae beyond stage IV. The monocultures of *R. reticulata* and the full mixed diet produced all naupliar stages with all other diets providing nauplii through to stage V. Table VI-25 presents the measurement of respiration rates of *E. modestus* larval stages feeding on the different algal diets. The measurement produced considerable variability with coefficients of variation ranging from 27% (Stage VI nauplii reared on *R. reticulata*) to 95% (Stage III nauplii reared on *S. costatum*).

Table VI-25. Respiration rates for *E. modestus* naupliar stages fed on different diets.

(a) mean (SD) respiration rates (nL oxygen animal^{-1} h^{-1}) for all feeding stages. Regression analysis shows exponent (±SE) of a power function fitted by least squares regression of Log10 transformed rates versus dry weights, and the standard error range (Int±SE) of the function constant (µg) along with correlation coefficient (r). SE = standard error of the difference

	Larval stages					Regression Analysis						
DIET	NII	NIII	NIV	NV	NVI	Slope	SE	Int-SE	Int+SE	r	df	p
Mix	4.35 (2.37)	5.08 (1.99)	6.01 (2.56)	3.95 (1.42)	4.49 (2.34)	-0.079	0.135	4.198	5.261	0.01	46	0.459
P.luth	1.57 (1.29)	3.12 (1.97)	4.00 (3.36)	6.78 (2.30)	-	1.076	0.273	1.510	2.173	0.291	25	0.004
R+S	2.14 (1.85)	5.52 (2.65)	3.99 (2.17)	-	-	0.734	0.329	2.377	3.266	0.110	28	0.073
R.ret	1.87 (0.93)	2.24 (1.23)	2.66 (1.52)	4.15 (1.27)	2.65 (0.68)	0.302	0.118	1.746	2.023	0.130	69	0.002
S.cost	0.61 (0.32)	1.56 (1.49)	4.78 (3.31)	6.51 (2.58)	-	1.894	0.273	0.750	1.074	0.565	25	0.001
T.chui	1.14 (0.84)	1.22 (0.90)	5.09 (2.48)	4.67 (2.21)	-	1.517	0.280	0.902	1.334	0.513	22	0.001

(Cont.)

(Cont. Table VI-25)

(b) Analysis of variance (diet x dry weight) comparing regressions for those diets producing significant Log/Log relationships (all from table (a) but the two mixtures).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
diet	3	0.6178	1.8348	0.6116	7.31	<0.001
Log Weight	1	4.7350	7.6549	7.6549	91.48	<0.001
Diet*LogWeight	3	3.2876	3.2876	1.0959	13.10	<0.001
Error	141	11.7984	11.7984	0.0837		
Total	148	20.4388				

(c) Comparisons between the average Log/Log regression slope or intercept (1.197± 0.125 & 0.131 ± 0.032, respectively) and the slopes/intercepts derived for each monoculture diet. (Difference is between individual slope/intercept and average slope/intercept, SE = standard error of the difference)

	Slope comparisons				Intercept comparisons			
Diet	Difference	SE	t	p	Difference	SE	t	p
P.lutheri	-0.1214	0.2331	-0.52	0.603	0.12712	0.05614	2.26	0.025
R.reticulata	-0.8955	0.1515	-5.91	<0.001	0.14240	0.04315	3.30	0.001
S.costatum	0.6971	0.2331	2.99	0.003	-0.17815	0.05614	-3.17	0.002
T.chuii	0.3197	0.2376	1.35	0.181	-0.09137	0.06329	-1.44	0.151

Given that dry weight nearly doubled at each larval stage it was expected that respiration rates would likewise show an increase from stage to stage but with the probability of following the surface area to volume law. As Table VI-25 shows, larvae on the fully mixed diet showed little change in average oxygen consumption rates from NII to NVI and the relationship between rate and dry weight (Log_{10} transformed) was not significant. The other mixed diet (*R. reticulata & S. costatum*) larvae did show a potential increase in oxygen consumption rate over the limited number of stages obtained (see Table VI-25) but within the variability of the data, no significant relationship (Log_{10} transformed) between rate and dry weight was obtained. All larvae fed on the monoculture diets did show significant, if occasionally variable, Log/Log relationships between consumption rate and dry weight (see Table VI-25).

Table VI-25(b) shows an analysis of variance which compares the Log/Log

regressions of oxygen consumption rates against dry weight for all the monoculture diets. The overall regression was significant ($F_{1,141} = 91.5$, $p < 0.001$) and the interaction term indicated at least one significant difference between slopes ($F_{df=3,141} = 13.1$, $p < 0.001$). The residuals from the fitted lines were approximately normally distributed (Anderson-Darling $A^2 = 0.67$, $p = 0.079$) but the residual variance for the larvae fed on *R. reticulata* was significantly lower than that for the other monoculture diets (Bartlett's $X^2 = 10.92$, $p = 0.012$).

Table VI-25(c) compares the individual regression slopes to the average of all 4 diets. The slopes for larvae fed on *P. lutheri* and *T. chui* (1.1 and 1.5 nl O_2 animal^{-1} h^{-1} μgDW^{-1}, respectively) do not differ significantly from the average (hence not from each other) and indicate a reasonably linear rise in oxygen consumption rate with dry weight (slopes not significantly different from 1). The larvae fed on *R. reticulata*, however, show a significantly lower slope (~0.3 nl O_2 animal^{-1} h^{-1} μgDW^{-1}, see Table VI-25(a)) than the average indicative of a reduction in weight specific respiration rate with dry weight. Examination of the averages (Table VI-25(a)), however, suggests that rates may rise slightly through the naupliar stages but possibly remain constant through the metanaupliar stages in a manner reminiscent of the response for larvae fed on the fully mixed culture (Table VI-25(a)). The slope for larvae fed on *S. costatum* (1.9 nl O_2 animal^{-1} h^{-1} μgDW^{-1}) is significantly greater than the average (and hence a slope of 1.197 nl O_2 animal^{-1} h^{-1} μgDW^{-1}) indicative of an exponentially increasing rate with dry weight. The average respiration rate of NV larvae fed on *S. costatum* was over ten times that of the NII larvae.

The intercepts of the Log/Log regressions (the power function constant in the arithmetic scale) indicate the estimated respiration rate for a larva of 1 μg in dry weight, i.e. late stage II early stage III *E. modestus* larvae (see Table VI-24 & Figure VI-26). Table VI-25(c) also shows comparisons between the average intercept and the individual intercepts (in the Log scale) of the regressions for larvae fed on each diet. The intercept for larvae fed *T. chuii* does not differ significantly from the average but that for larvae fed *S. costatum* is significantly lower than the average. The SE ranges for these two groups of larvae overlap, in the arithmetic scale, indicating a range of between 0.75 and 1.33 nl O_2 animal^{-1} h^{-1}. The intercepts for larvae fed *P. lutheri* and *R. reticulata* were very similar and both were significantly above the average. The SE

range (in the arithmetic scale) for these two groups of larvae was set by the fewer observations obtained for those fed on *P. lutherii* (see Table VI-25a)) indicating a range of between 1.5 and 2.2 nl O_2 animal^{-1} h^{-1}. The geometric mean values (comparable to the intercepts estimated above) of respiration rates for NII larvae fed on the two mixed cultures (no significant relationship with dry weight) were much higher than the intercepts for any of the functions fitted for the monoculture fed larvae ($R+S = 3.02$, mixed = 4.4 nl O_2 animal^{-1} h^{-1}) with SE ranges from 2.5 to 3.7 nl O_2 animal^{-1} h^{-1} for larvae fed a mixture of *R. reticulata* and *S. costatum* and 3.9 to 5.0 nl O_2 animal^{-1} h^{-1} for larvae fed on the full algal mixture. Thus larvae at an equivalent stage (and weight) consumed twice as much oxygen h^{-1} when fed on algal mixtures than when fed on monocultures of *P. lutheri* and *R. reticulata* but those fed monocultures of *T. chuii* and *S. costatum* consumed half as much again.

VI-3.4 Oxygen consumption of *Elminius modestus* larvae feeding and non-feeding on *Rhinomonas reticulata*

Generally, the average respiration rates of fed *E. modestus* larvae were higher than those of unfed animals Table VI-26 (a)). Also, rate generally increased with stage (hence dry weight) for both fed and unfed larvae although the rate of increase is slower in the later naupliar stages. As previously noted, respiration rates showed high variability ranging from a cv of 26% (Fed NVI) to 72% (Unfed NIV) although variability between treatments was roughly comparable.

The regression analysis of Log_{10} transformed oxygen consumptions at each feeding stage were compared (Table VI-26(a)). Again, anticipating a power function relationship between consumption rate and dry weight the result was analysed by regression. Results for NVI were omitted from both treatments since the observations did not fit the power function very well. It appears that respiration rates dropped in the final naupliar stage by an amount in excess of that expected by proportionality with weight.

Table VI-26. Respiration rates of *E. modestus* nauplii reared on *R. reticulata* cultures against stage. Nauplii were either fed or deprived of food (unfed) prior and during the respiration measurements.

(a) mean (SD below) respiration rates (nl oxygen animal^{-1} h^{-1}) for all feeding stages. Regression analysis shows: the exponent (±SE) of a power function fitted by least squares regression of Log_{10} transformed rates versus dry weights; the standard error range (±SE) of the function constant (int (µg)) along with the correlation coefficient (r).

	means (and standard deviation)					Regression analysis							
Food	NII	NIII	NIV	NV	NVI	Slope	SE	int	-SE	+SE	r	df	p
unfed	0.91 0.63	1.28 1.06	1.81 1.30	1.47 0.55	1.62 0.82	0.596	0.187	0.83	0.97	1.10	0.394	42	0.008
fed	1.85 0.85	2.24 1.23	2.60 1.52	4.15 1.27	2.65 0.68	0.465	0.163	1.84	1.89	2.05	0.435	55	0.001

(b) Analysis of variance table comparing Log/Log regressions for fed and unfed larvae. Rates for stage VI nauplii have been excluded since they did not fit the trends shown by the rest of the consumption rates.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
food presence	1	1.69906	1.44954	1.44954	21.50	<0.001
LogWeight	1	1.21678	1.23560	1.23560	18.33	<0.001
food*LogWeight	1	0.01893	0.01893	0.01893	0.28	0.597
Error	101	6.80950	6.80950	0.06742		
Total	104	9.74427				

In addition, Table VI-26(a) shows that the slopes (exponents of the power function) for both treatments were quite similar with equivalent standard errors. The constant of the power functions for unfed larvae was less than half that of the fed larvae (Table 4(a), expressed in the arithmetic scale) with no overlap in SE range. Table 4(b) compares the two regression lines by analysis of variance. The residuals from the lines were approximately normal ($A^2 = 0.735$, $p = 0.054$) and residual variances approximately equal (Hartley's $F = 1.71$ $p = 0.054$). The interaction term was not significant ($F_{1,101} = 0.28$, $p = 0.597$) indicating no significant difference in slope between treatments. The effect of food, however, was highly significant ($F_{1,101} = 21.5$, $p < 0.001$) showing that the regression intercept for fed larvae was significantly greater than that for unfed larvae.

Thus for both treatments the power function relating rate to dry weight has an

exponent of 0.53 (± 0.123, SE) on average demonstrating a reducing relative oxygen consumption rate in larger nauplii. Furthermore, the average slope does not differ significantly ($t = 1.125$, $df = 101$, $p = 0.263$) from the 0.67 exponent predicted from surface area/volume theory. However, throughout the range of weights (hence stages) analysed, respiration rates of fed nauplii were 2.2 times higher on average.

VI.4. Discussion

Oxygen consumption reflects the energy spent by organisms under any given circumstance. *Elminius modestus* NII larvae have a non-feeding rate approximately of 0.3 – 0.7 nL O^2 $animal^{-1}$ $hour^{-1}$, while nauplii feeding on *T. chui* or *S. costatum* have a slightly elevated rate, suggesting these do not extract a great amount of nutrients from these algae. Larvae fed on *P. lutheri* and on *R. reticulata*, particularly at the early naupliar stages, showed a significantly higher respiration rate than non-fed ones, indicating a higher metabolic activity on these diets. And finally, the vastly increased and stable rates for the algal mixtures suggest a considerable extraction of nutrients but potentially heightened over all digestive mobilisation needed to cope with the variety in the diet.

Respiration rates result from different physiological processes including swimming activity, capture of food, and digestive processes which are offset by the energy obtained from assimilation. Contributions to oxygen consumption from the different components, i.e. the energy budget, are not examined within the present study; nevertheless, it is clear that *E. modestus* nauplii consume more than twice the amount of oxygen when in presence of food.

Although the range of weights differed between diets, there is little evidence that respiration rates followed the surface/volume law. As shown by Equation VI-1, relative growth is higher during later metamorphosis, which would implicate a higher respiration rate during stages NIV-NVI (if an arithmetic relationship was the only determining factor). However, in the diets where these stages were tested, this was not the case, indicating possible physiological differences between nauplii and metanauplii and therefore a different balance between food, growth and metabolism.

Except for the rates presented by Jorgensen & Vernberg (1982) for *Balanus*

eburneus, the specific weight respiration rates of which varied from 18.08 (NI) to 33.45 (NVI) $nLO_2\ \mu gDW^{-1}\ hour^{-1}$, the range rates established for feeding larvae within the present study conform to those presented by Lucas (1980) and Harms (1987) for *S. balanoides* and *E. modestus* respectively. At 18°C, *E. modestus* seem to change the weight-specific respiration activity before and after metamorphosis into NIV (Harms 1987), following a decrease in the energy loss by metabolism. The assumption that metanauplii have a better energetic efficiency than nauplii would have to be verified with further experimentation on assimilation efficiency and analysis of digestive enzymes at all larval stages of these barnacles.

Considering unfed larvae, the low oxygen consumption of NII are comparable to those found by Collis and Walker (1995, and cited reviews), on the parasitic lecithotrophic *Sacculina carcini* larvae (from 0.95 (male NII) to 1.54 (female NIII) $nl\ O_2\ \mu gDW^{-1}\ h^{-1}$). Without the input of energy, larvae adapt a conservative basal metabolism. This is likely to be determined by a regulatory mechanism of energy expenditure according to the remaining energy, as shown by the drop in oxygen consumption of unfed NII larvae after 8 hours from hatching.

Oxygen consumption of previously fed larvae decreases by more than half when food is absence, as shown by the results between feeding and starving nauplii at all stages. After transference into UV filtered seawater, hence clear from food, larval respiration did not change over time, which suggests an extraordinary short period in which these animals digest food (Specific Dynamic Action). Thus, the differences between feeding and starving animals result from the capture of food particles, i.e. *E. modestus* nauplii spend a great proportion of their energy obtaining algal cells and that the actual metabolism involved in the other physiological processes is comparatively low.

Table VI-27 summarises the mean values of oxygen consumption of *E. modestus* at different larval stages obtained in different suspensions (feeding and non-feeding). Respiration served as a metabolism measurement of these animals regarding the given environmental conditions and analysed with swimming activity and feeding rates (discussed in previous chapters), provided clues on barnacle larval physiological processes and adaptations. This integrative approach will be discussed in the following chapter, General Discussion.

Table VI-27. Mean respiration rates (nLO_2 animal^{-1}hour^{-1}) of *E. modestus* larvae (nauplius I-VI). Larvae were fed on *R. reticulata* (100 cells µL^{-1}) prior to measurements and transferred to different mono-algal suspensions of the same final cell density or to UV-filtered seawater (UVFSW). Mixed cultures (MIX) were of *R. reticulata*, *C. neogracilis*, *P. lutheri*, *S. costatum*, and *T. chui*, at equal proportions (a) oxygen consumption immediately after hatching (b) Resp. rate = -0.0076 ($time_{minutes}$) + 4.343 (c) starved since hatching.

		Respiration rate (nLO_2 animal^{-1} h^{-1})						
Stage	Dry Weight (µg animal^{-1})	UVFSW	*R. reticulata*	*P. lutheri*	*S. costatum*	*T. chui*	*R. reticulata* + *S. costatum*	MIX
NI	0.521	4.34[ab]	-	-	-	-	-	-
NII	0.751	0.91	1.87	1.57	0.61	1.14	2.14	4.35
		0.54[c]	-	-	-	-	-	-
NIII	1.107	1.28	2.24	3.12	1.56	1.22	5.52	5.08
NIV	1.898	11.8	2.66	4.00	4.78	5.09	3.99	6.01
NV	3.001	1.47	4.15	6.78	6.51	4.67	-	3.95
NVI	5.488	1.62	2.65	-	-	-	-	4.49

VII General Discussion and Conclusions

Cirripedia larvae respond to the abundance of food in the environment through feeding and consequent physiological effects, both qualitatively and quantitative, on swimming behaviour and metabolism. The current study investigates aspects of the physiology in relation to the feeding process of two species of barnacle larvae.

Generally, the capture of particles by herbivorous/omnivorous zooplankton depends on the particle size, the surface area and shape of the "filtering" appendages, the physical properties of the water (e.g. density and viscosity) and the limb beat rate, which is associated with the swimming activity of the larvae (Hart & Strathmann 1995). Within the range of algal cells ingested, the size and volume of particles captured by the larvae increase with development stage: as larvae get larger, so do the surface area of the limbs hence the volume of water that can be handled. Mediated by chemical cues and mechanical stimulation from the algae (Walker *et al.* 1987), ciiripede larvae adjust their limb rate in relation to algal abundance (Yule 1982), balancing the energy they expend in capturing algae with the potential energy return from the food captured.

During the current study, *Pollicipes pollicipes* nauplii ingested higher volumes of the larger *Tetraselmis chui* during the later developmental stages (Chapter III). Furthermore, *Elminius modestus*, showed a distinct preference for *Skeletonema costatum* over *Rhinomonas reticulata,* possibly because the small cells of the diatom can form chains some 3 – 4 times larger than the single-celled flagellate (Chapter IV). Nevertheless, size alone is probably not the only determinant of feeding rates and diet selection of barnacle larvae. The inferred 'condition' of the phytoplankton diet played a major a role in determining survival and development throughout the experiments on *P. pollicipes* and *E. modestus* (Chapters III and IV). Food culture health might also have been reflected in larval metabolism where low oxygen consumption rates were measured for larvae fed *S. costatum* cultures, which were later indicated to have been of questionable quality or even toxic. Furthermore, the lack of inherent nutritional quality of otherwise healthy cultures may also be inferred from the total lack of development of later stages of *P. pollicipes* when fed *T. chui* even though the algae sustained development through the early larval stages. The adequacy of a diet for larval growth

and survival had already been found to change throughout larval development (*E. modestus*, Stone 1988; *Balanus amphitrite*, Desai & Anil 2004; *Penaeus monodon*, Kurmaly *et al.* 1989b; *Sparus aurata*, Cañavate & Fernández-Díaz 2001) and this is sustained by the results found in present experiments on larval behaviour and respirometry.

The current results also show that barnacle larvae are not simply automatic devices ingesting particles indiscriminately. The significantly lower swimming speeds of larvae measured in the absence of food particles compared to those in the presence of food particles bears witness to an energy saving response (Walker *et al.* 1987). The significantly reduced oxygen consumption rates of larvae measured without algae in comparison to those with algae (Chapter VI) confirms that swimming energy loss is a significant proportion (80%) of total metabolism. Since the response is evident within 5 minutes of transfer of NII *E. modestus* from food to food-free conditions (Chapter V), it indicates that the change in swimming speed is a rapid mechanism which conserves energy in what is known to be a patch food environment (Cassie 1959, Legendre & Demers 1984, Mackas *et al.*1985).

The prompt behavioural and metabolic changes of *E. modestus* to the absence of food could be associated with the extensive spawning season of this barnacle in North Western Europe. The adults release the larvae practically throughout the year, which suggests that in order to survive during the nutritionally dilute winter months, these nauplii probably have to cope with periods of no food availability adequately enough to retain and build sufficient energy reserves for successful settlement. The effect of 28 hours of food deprivation from immediately after hatching does not seem to alter swimming performance of *E. modestus* larvae. Oxygen consumption rates, however, decreased to a minimal level after 8 hours without food. The minimal rates achieved ($0.721\ nLO_2\ \mu gDW^{-1}\ hour^{-1}$) were equivalent to those measured for *S. balanoides* cyprids ($1.17\text{-}0.66\ nLO_2\ \mu gDW^{-1}\ hour^{-1}$) which were considered by Lucas *et al.* (1979) to be one of the lowest routine rates recorded for marine organisms and reflected the need for an extended period of energy conservation in the lecithotrophic settlement stage. The low rate also approximates that of the nauplii of lecithotrophic species, e.g. *Sacculina carcini* (Collis & Walker 1995).

For newly metamorphosed *E. modestus* NII larvae, it is suggested that yolk reserves carried through to the feeding NII stage are sufficient to maintain larval activity for at least 28h should they hatch into water with no food available. The high initial respiration rates, measured in the current study, undoubtedly reflect high metabolic requirements of the non-feeding NI larvae (less than 4 hours after hatching) preparing for or undergoing the rapid moult to the feeding NII stage. The majority of larvae will have moulted to NII within 2-3 hours of hatching. The reduction in oxygen consumption rates which occurs in NII larvae after ~8 h clearly conserves energy to extend the hunt for food but it does not occur at the expense of swimming speeds. Since maintaining high swimming speeds appears energetically expensive (see above), it is possible that reduced respiration rates may reflect a decrease in the development rate during NII which frees energy reserves for the more pressing need to find food (decapod larvae, Knowlton 1974, Anger & Dawirs 1981).

The effect of starvation on larval development and survival depends mainly on the amount of reserves accumulated by the larvae at the time of starvation, and consequently on the length of the starvation period. If larvae carry enough stored energy reserves they can survive starvation for longer and extend development time by retarding the next moult or by lengthening the duration of subsequent stages in order to compensate for previous losses in biomass and energy (Boidron-Métairon 1995). The "point-of-no-return" (Anger and Dawirs 1981) or "ultimate recovery hour" (Desai & Anil 2004) can be defined as the maximal starvation time after which larvae will cease to develop and die and that time depends on several factors. Nauplii starving at higher temperatures suffer from faster degradation of energy reserves and higher metabolic demands and therefore lower survival (*Balanus amphitrite*, Desai & Anil 2004). In *B. amphitrite*, the absence of food during the earlier larval stages seems to inflict a greater detriment than if food deprivation occurs later in development. Although Lang & Marcy (1982) indicated that survival potential of *Balanus improvisus* decreases 24 hours after hatching, mortality of all the individuals was only observed after 7 days of experiment without food. Furthermore, in *E. modestus*, Harms (1988) observed that NII larvae starved for the same period length (7 days) could still develop to cyprid, if fed an adequate diet immediately after the starvation period. Thereby, the quality of the food encountered after food deprivation will determine the ability of the larvae to resume normal development (Desai & Anil 2004). In the current study, NII *E. modestus* deprived of food for 12 - 14 hours showed no significant differences in

feeding rate or dietary selectivity from larvae which had been continuously fed from hatch. No obvious compensatory strategy for the energy loss incurred through the deprivation period was thus evident. Either the larvae always attempt to feed at a maximal rate whenever food is available, irrespective of previous feeding history, or the deprivation period was too short for the larvae to require a compensatory strategy.

During development, the dietary requirements of the nauplii appear to change. Thus, survival rates of *P. pollicipes* larvae fell considerably after the moult from NIII to NIV when cultured using *S. costatum* and *T. chui*, which did not happen when the larvae were cultured with *R. reticulata* or an algal mixture. In these cases, the metanauplii of *E. modestus* exhibited a similar weight specific respiration rate at each nauplius stage. However, those larvae fed either *S. costatum* or *P. lutheri* exhibited increasing weight specific respiration rates with stage, indicative of an increased metabolic cost of food capture or digestion. During culture, *R. reticulata* proved more effective in growing *E. modestus* larvae than *S. costatum*, and *P. lutheri* proved a particularly poor rearing food

The swimming speeds of NVI larvae were significantly lower than those for NV. Such might result from the increased size of the NVI moving them into a hydrodynamic situation where form drag rather than friction drag is more important in resistance to motion. The change in shape from NVI to cyprid clearly indicates a hydrodynamic influence since the cyprid shape is universally more 'streamlined' than the nauplius throughout the Cirripedia. However, other more physiological changes may well be occurring in the later naupliar stages which relate to swimming through feeding rates. The fact that weight specific respiration rate does not drop at NVI even though average swimming speeds do suggests an increase in non-swimming related metabolic activity. It is known that digestive protease activity of NIV-NV *E. modestus* was lower than that of NI-NIII (Le Vay *et al.* 2001). These authors interpreted the difference as corresponding to a switch from herbivory to omnivory in later larval development. It may, however, reflect potentially lower feeding rates or reduced digestive efficiency during the metanaupliar stages. During the culture of *S. balanoides* (Tighe-Ford *et al* 1970, Yule *pers. comm.*) and *E.modestus* (Yule *pers. comm.*) larvae, a reduction in the density of food algae is often undertaken at stage V & VI to aid the success of the culture. The limbs of the later metanaupliar stages often become clogged with food when algae are present at too high a density, and survival rates decline. Reduced feeding levels appear sufficient to promote development yet prevent limb

clogging. Cyprids do not feed and the bulk of the storage material for cyprid survival has presumably lain down prior to the moult to NVI. The focus of metabolic activity at NVI, and possibly NV, thus appears to be development (compound eyes, thoracic appendages, maxillae etc, all of which are clearly visible, though under-developed, at NV) rather than feeding for storage. Feeding rates may thus be reduced, conserving energy expenditure on food capture, but metabolic rates remain high (oxygen consumption) as development proceeds in preparation for the final pelagic moult.

The timing of these development events coincides with the hydrodynamic imperative of the shape change. This is necessary to offset the increasing form drag experienced with an increase in size and the necessity of cyprid speed to initially attach to a surface and then remain attached to it in the face of potentially high current speeds impinging on the larvae. The Cirripedia, as a group, are the only truly sessile crustaceans. Whether free living or parasitic, the life history of all those which broadcast larvae for dispersal is essentially the same, ending with a major shape change for the final larval stage which closes the lifecycle by performing the transition from planktonic to benthic. It would appear that a major evolutionary driving force behind the timing of development and the partitioning of function between larval stages may well be hydrodynamic as well as the effect of viscosity in determining resistance in relation to size and speed.

In larval feeding experiments, account should be taken of 1) the origin of the nauplii, 2) food quality, 3) food quantity, 4) the algal growth phase, 5) the culture conditions (temperature, salinity, light, etc.), and 6) the cleanliness of the cultures (after Harms 1988). During the present study, the application of a rotating wheel submersed in a temperature-controlled tank and the careful manipulation of the animals assisted in the maintenance of actively swimming larval cultures. The density of *R. reticulata* food was maintained to produce maximal feeding without clogging appendages. Clogged limbs in excessively dense algal cultures are a major cause of larval mortality. Maintaining good quality *S. costatum* was a major difficulty during the experiments with *E. modestus*, and although experiments were repeated whenever algal condition was considered 'suspect' through microscopic observation of the cells, the 'average' quality of *S. costatum* over the time course of rearing a culture may not have been as good as those that led Harms (1988) to declare *S. costatum* as the most appropriate diet for culturing *E. modestus* nauplii. Nevertheless, the results of the shorter term experiments with *S. costatum* remain comparable. NII larvae consumed less oxygen

when feeding on the diatom than when feeding on *R. reticulata*, suggestive of either lower energy expenditure in feeding on or lower energy expenditure in digestion (lower digestion percentage or higher digestive efficiency) of the diatom. A clear ability to select preferentially *S. costatum* in mixed culture with the flagellate was shown, based on cell volume densities. At 100 cells μL^{-1} (density used in respirometry), the ingested volume of *S. costatum* was approximately 25% of that ingested of *R. reticulata* (Table IV-19). Thus the lower respiration rate may reflect a reduced energy loss through digestion since the ingested quantity was lower, i.e. apparent specific dynamic action proportional to ingestion rate. Clearance rates were higher at the lowest cell densities used but relatively stable over a wide range of cell volume densities. At worst, the high clearance rates would imply an increase in respiration rate (more activity in handling more water) for larvae feeding on *S. costatum* at a lower cell volume density than when feeding on *R. reticulata*. On balance, it is more likely that the difference in respiration rates is related to volume ingested through digestive heat and hence, possibly, the first demonstration of specific dynamic action in barnacle larvae.

It has been suggested that feeding preferences demonstrated in cirripede larvae can be associated with their geographic distribution (Moyse 1963, Stone 1989, and results in this study). Interesetular distances appear wider in cold water balanoids (*Semibalanus balanoides*, *Balanus crenatus*), which feed mainly on diatoms while warmer water species (*B. perforatus*, *Chthamalus montagui*, *C. stellatus*) tend to have fine-meshed setae and additional fringes of setules along the outer fringe of the antennae and feed more readily on small flagellates. Flagellates tend to be more abundant in generally warmer waters (review *in* Raymont 1983, Varella 2005). *E. modestus* and *P. pollicipes* are able to feed and develop on both diatoms and flagellates, although larger particles appear preferable. *E. modestus* breed continuously throughout the year hence experience a wide range of phytoplankton communities. *P. pollicipes* have a more resitricted breeding season (Cardoso & Yule 1995) but are mostly found in areas of considerable upwelling which are characterised by high phytoplankton diversity (Moita *el al.* 2003).

For vulnerable meroplankton, development times should be minimised, in order to avoid predation and accidental mortality, but balanced, in barnacle larvae, with maximisation of energy storage to allow lecithotrophic cypris larvae enough time to find an adequate settlement location. The variability in clearance rates, swimming

speeds, respiration rates and swimming behaviour are characteristic of a "plastic population" (after Margalef 1997), i.e. a group of individuals belonging to the same species that, as a whole, have wide tolerance levels to different stress factors. As discussed, for their abundance and distribution, barnacle larvae can have a major impact on local phytoplankton assemblages. Along with intrinsic characteristics of phytoplankton species, such as cell division rates and specific sinking rates, and accompanied by physical processes like turbulent diffusion, this may be influential in structuring marine communities. Mixing and upwelling currents allow the regeneration of nutrients and the sustainability of marine pelagic systems, which contribute with one fourth of the total productivity of the biosphere (Margalef 1997). The ecological dynamics assume transference of energy within organisms, which creates diverse physico-biological conditions and defines complex food-web relationships. The details of specific feeding rates, dietary preference, physiological processes, swimming behaviour and the physics that drive evolutionary change, aspects of which have been addressed in the current work, indicate how barnacle larvae may exert their influence on phytoplankton communities.

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APPENDIX

The following tables contain the original data derived from the experiments undertaken during this study and refer to the following sections:

III.1 – Larval measurements of *Pollicipes pollicipes* (μm), where larval stage 1 to 6 is nauplis I to VI and L is body total length, CSL carapace shield length, W greatest width, GL gut total length, and GW gut greatest width.

stage	L	CSL	W	GL	GW
1	252.0	*	132.0	*	*
1	214.2	*	153.0	*	*
1	234.6	*	142.8	*	*
2	387.6	*	214.2	61.2	71.4
2	384.0	*	220.0	64.0	36.0
2	336.0	*	216.0	68.0	44.0
2	387.6	*	224.4	71.4	40.8
2	387.6	*	224.4	71.4	40.8
2	397.8	*	224.4	71.4	40.8
2	384.0	*	220.0	76.0	52.0
2	387.6	*	214.2	81.6	40.8
2	397.8	*	224.4	81.6	81.6
2	368.0	*	248.0	84.0	60.0
2	377.4	*	224.4	91.8	81.6
2	357.0	*	204.0	91.8	71.4
2	397.8	*	214.2	102.0	81.6
2	336.6	*	224.4	122.4	51.0
2	397.8	*	234.6	142.8	61.2
2	377.4	*	204.0	153.0	40.8
2	377.4	*	224.4	153.0	61.2
2	397.8	*	224.4	153.0	61.2
2	346.8	*	214.2	*	*
2	377.4	*	204.0	*	*
2	377.4	*	214.2	*	*
2	377.4	*	244.8	*	*
2	377.4	*	204.0	*	*
2	387.6	*	183.6	*	*
2	387.6	*	255.0	*	*
2	397.8	*	234.6	*	*
2	397.8	*	214.2	*	*
2	397.8	*	234.6	*	*
2	408.0	*	234.6	*	*
2	408.0	*	224.4	*	*
2	459.0	*	244.8	*	*
2	332.0	*	200.0	*	*
2	285.6	*	183.6	*	*
2	336.6	*	142.8	*	*
3	428.4	*	255.0	61.2	61.2
3	418.2	*	234.6	61.2	61.2
3	408.0	*	255.0	91.8	61.2
3	428.4	*	275.4	91.8	61.2
3	428.4	*	255.0	91.8	102.0
3	397.8	*	234.6	91.8	91.8
3	408.0	*	244.8	91.8	61.2
3	408.0	*	255.0	91.8	61.2
3	377.4	*	255.0	102.0	61.2
3	428.4	*	255.0	112.2	71.4
3	448.8	*	244.8	102.0	61.2
3	408.0	*	244.8	122.4	102.0
3	408.0	*	244.8	102.0	122.4
3	418.2	*	265.2	132.6	71.4
3	448.8	*	244.8	102.0	102.0

stage	L	CSL	W	GL	GW
3	449.8	*	275.4	112.2	122.4
3	459.0	*	275.4	102.0	61.2
3	408.0	*	244.8	122.4	71.4
3	408.0	*	234.6	112.2	102.0
3	428.4	*	234.6	112.2	81.6
3	438.6	*	234.6	132.6	91.8
3	418.2	*	265.2	132.6	81.6
3	408.0	*	255.0	*	*
3	408.0	*	234.6	*	*
3	418.2	*	255.0	*	*
3	428.4	*	244.8	*	*
3	438.6	*	265.2	*	*
3	438.6	*	275.4	*	*
3	448.8	*	255.0	*	*
3	448.8	*	275.4	*	*
3	448.8	*	255.0	*	*
3	408.0	*	265.2	*	*
3	408.0	*	265.2	*	*
3	418.2	*	255.0	*	*
3	418.2	*	265.2	*	*
3	418.2	*	265.2	*	*
3	418.2	*	295.8	*	*
3	428.4	*	265.2	*	*
3	428.4	*	275.4	*	*
3	428.4	*	265.2	*	*
3	428.4	*	255.0	*	*
3	438.6	*	255.0	*	*
3	438.6	*	265.2	*	*
3	438.6	*	255.0	*	*
3	438.6	*	255.0	*	*
3	448.8	*	275.4	*	*
3	459.0	*	275.4	*	*
4	479.4	*	265.2	*	*
3	380.0	*	252.0	*	*
3	364.0	*	204.0	*	*
3	418.2	*	255.0	*	*
3	387.6	*	193.8	*	*
4	448.8	306.0	275.4	102.0	51.0
4	469.2	306.0	275.4	102.0	51.0
4	418.2	285.6	255.0	112.2	30.6
4	469.2	306.0	285.6	112.2	61.2
4	448.8	265.2	255.0	122.4	40.8
4	408.0	275.4	285.6	122.4	61.2
4	408.0	295.8	316.2	122.4	61.2
4	469.2	316.2	306.0	132.6	51.0
4	*	*	295.8	142.8	61.2
4	418.2	244.8	255.0	153.0	71.4
4	418.2	265.2	265.2	163.2	81.6
4	418.2	275.4	295.8	173.4	81.6
4	448.8	306.0	285.6	183.6	91.8
4	448.8	295.8	306.0	183.6	81.6
4	479.4	306.0	306.0	193.8	91.8
4	408.0	306.0	295.8	204.0	102.0
4	*	*	285.6	214.2	112.2
4	469.2	326.4	316.2	224.4	102.0
4	*	326.4	316.2	234.6	132.6
4	479.4	316.2	275.4	244.8	142.8
4	448.8	285.6	316.2	255.0	142.8
4	448.8	*	285.6	*	*

stage	L	CSL	W	GL	GW
4	*	234.6	234.6	*	*
4	*	316.2	336.6	*	*
4	452.0	280.0	*	*	*
4	438.6	295.8	*	*	*
4	469.2	306.0	*	*	*
4	418.2	255.0	*	*	*
4	428.4	255.0	*	*	*
4	408.0	244.8	*	*	*
5	489.6	357.0	367.2	*	*
5	489.6	306.0	306.0	102.0	81.6
5	408.0	285.6	397.8	*	*
5	499.8	346.8	367.2	*	*
5	408.0	275.4	316.2	142.8	142.8
5	469.2	326.4	357.0	153.0	91.8
5	479.4	326.4	357.0	153.0	91.8
5	479.4	326.4	357.0	183.6	91.8
5	479.4	326.4	346.8	193.8	102.0
5	479.4	346.8	316.2	193.8	102.0
5	459.0	306.0	326.4	204.0	102.0
5	469.2	316.2	357.0	204.0	102.0
5	469.2	316.2	357.0	*	*
5	499.8	336.6	336.6	204.0	102.0
5	530.4	357.0	336.6	204.0	91.8
5	469.2	316.2	346.8	204.0	102.0
5	469.2	367.2	357.0	*	*
5	479.4	326.4	357.0	*	*
5	479.4	336.6	367.2	204.0	102.0
5	489.6	336.6	326.4	224.4	132.6
5	469.2	326.4	357.0	*	*
5	469.2	316.2	357.0	244.8	122.4
5	479.4	326.4	357.0	244.8	122.4
5	479.4	326.4	367.2	275.4	112.2
5	428.4	*	387.6	275.4	153.0
5	479.4	316.2	336.6	*	*
5	479.4	316.2	326.4	275.4	112.2
5	479.4	326.4	367.2	285.6	122.4
5	418.2	*	387.6	*	*
5	469.2	336.6	336.2	285.6	122.4
5	479.4	326.4	357.0	285.6	153.0
5	499.8	357.0	367.2	*	*
5	520.2	357.0	367.2	*	*
5	346.8	377.4	*	*	*
5	428.4	316.2	316.2	*	*
5	438.6	326.4	306.0	*	*
5	448.8	316.2	367.2	*	*
5	448.8	326.4	336.6	*	*
5	458.0	295.8	316.2	*	*
5	459.0	306.0	316.2	*	*
5	459.0	336.6	316.2	*	*
5	469.2	306.0	316.2	*	*
5	469.2	316.2	326.4	*	*
5	469.2	316.2	377.4	*	*
5	479.4	316.2	316.2	*	*
5	479.4	357.0	326.4	*	*
5	489.6	306.0	316.2	*	*
5	489.6	316.2	326.4	*	*
5	489.6	336.6	336.6	*	*
5	489.6	336.6	326.4	*	*
5	499.8	326.4	357.0	*	*
5	499.8	336.6	326.4	*	*

stage	L	CSL	W	GL	GW
5	499.8	336.6	346.8	*	*
5	530.4	346.8	326.4	*	*
5	*	306.0	275.4	*	*
5	*	377.4	255.0	*	*
5	*	397.8	265.2	*	*
5	*	397.8	265.2	*	*
6	499.8	357.0	346.8	183.6	91.8
6	499.8	326.4	367.2	193.8	91.8
6	510.0	357.0	357.0	*	*
6	520.2	377.4	346.8	193.8	112.2
6	489.6	346.8	367.2	*	*
6	499.8	357.0	357.0	204.0	102.0
6	591.6	418.2	469.2	204.0	112.2
6	540.6	387.6	397.8	214.2	102.0
6	499.8	346.8	346.8	234.6	91.8
6	540.6	387.6	397.8	234.6	142.8
6	499.8	367.2	346.8	244.8	142.8
6	499.8	346.8	357.0	244.8	204.0
6	520.2	346.8	367.2	244.8	153.0
6	520.2	367.2	377.4	*	*
6	530.4	408.0	377.4	255.0	153.0
6	601.8	438.6	428.4	*	*
6	540.6	367.2	357.0	255.0	153.0
6	581.4	428.4	377.4	265.2	153.0
6	581.4	428.4	408.0	275.4	183.6
6	612.0	438.6	397.8	275.4	153.0
6	510.0	367.2	367.2	285.6	153.0
6	520.2	357.0	357.0	*	*
6	520.2	367.2	397.8	*	*
6	581.4	418.2	408.0	295.8	153.0
6	561.0	408.0	377.4	306.0	153.0
6	540.6	397.8	418.2	*	*
6	561.0	438.6	387.6	316.2	122.4
6	510.0	377.4	397.8	*	*
6	550.8	397.8	367.2	*	*
6	550.8	408.0	418.2	*	*
6	550.8	428.4	448.8	*	*
6	561.0	397.8	357.0	*	*
6	561.0	397.8	397.8	*	*
6	561.0	397.8	387.6	*	*
6	561.0	408.0	397.8	*	*
6	571.2	408.0	469.2	*	*
6	581.4	418.2	377.4	*	*
6	581.4	418.2	377.4	*	*

III.2 – Feeding rates of *Pollicipes pollicipes* on algal monocultures

Larval Stage	Algal Species	Time Hours	Vial volume µL	Larval N	Vial Number	algal densities (cells µL^{-1}) control initial	control final	experimental initial	experimental final
NII	*Isochrysis galbana*	7.3333333	30000	15	1	73.9	77.1	73.9	47.8
					2	73.9	58.8	73.9	41.7
					3	65.3	63.5	65.3	49.9
					4	73.2	57.5	73.2	52.4
					5	69.7	56.0	69.7	48.3
					6	69.4	61.3	69.4	50.4
					7	66.7	58.1	66.7	37.5
	Phaeodactylum tricornutum	7.25	30000	15	1	100	83.3	100	80.7
					2	100	79.9	100	67.9
					3	100	84.2	100	83.0
					4	100	76.5	100	73.8
					5	100	77.6	100	71.4
					6	100	81.8	100	70.4
					7	100	73.8	100	67.9
	Tetraselmis chui	7.5	30000	15	1	100	152.0	100	140.0
					2	100	150.0	100	145.0
					3	100	129.0	100	108.0
					4	100	149.0	100	125.4
					5	100	193.0	100	163.0
					6	100	139.0	100	125.0
					7	100	148.0	100	134.0
	Skeletonema costatum	6.87	30000	15	1	100	121.0	100	63.7
					2	100	100.5	100	97.5
					3	100	127.0	100	92.5
					4	100	93.0	100	63.5
					5	100	104.5	100	93.5
					6	100	122.0	100	85.4
					7	100	134.0	100	80.0

Larval Stage	Algal Species	Time Hours	Vial volume µL	Larval N	Vial Number	algal densities (cells µL^{-1}) control initial	control final	experimental initial	experimental final
NIII	*Isochrysis galbana*	6.75	30000	15	1	100	76.5	100	43.0
					2	100	44.0	100	34.0
					3	100	32.0	100	20.5
					4	100	24.5	100	22.0
					5	100	27.5	100	19.0
					6	100	50.5	100	33.5
					7	100	50.5	100	38.5
	Phaeodactylum tricornutum	6.8	30000	15	1	100	20.0	100	21.5
					2	100	26.0	100	18.5
					3	100	29.0	100	22.5
					4	100	10.0	100	12.0
					5	100	15.0	100	16.5
					6	100	35.0	100	31.0
					7	100	32.0	100	30.0
	Tetraselmis chui	7.01	30000	15	1	100	186.0	100	171.5
					2	100	189.5	100	174.5
					3	100	205.0	100	182.0
					4	100	168.5	100	165.0
					5	100	163.0	100	149.0
					6	100	162.5	100	138.5
					7	100	230.0	100	199.0
	Skeletonema costatum	5.4	30000	15	1	100	113.0	100	90.5
					2	100	139.5	100	96.5
					3	100	132.5	100	81.0
					4	100	96.5	100	83.4
					5	100	84.8	100	84.5
					6	100	142.0	100	83.5
					7	100	112.3	100	91.5

Larval Stage	Algal Species	Time Hours	Vial volume µL	Larval N	Vial Number	algal densities (cells µL^{-1}) control initial	control final	experimental initial	experimental final
NIV	*Isochrysis*	7.58	30000	15	1	100	26.3	100	11.5
	galbana				2	100	64.5	100	41.6
					3	100	38.5	100	19.0
					4	100	41.0	100	26.5
					5	100	64.0	100	41.6
					6	100	46.2	100	32.0
					7	100	41.6	100	23.5
	Tetraselmis	7.83	30000	15	1	100	180.0	100	164.0
	chui				2	100	172.0	100	152.0
					3	100	145.5	100	139.0
					4	100	148.5	100	145.5
					5	100	191.0	100	161.0
					6	100	165.0	100	141.0
					7	100	164.0	100	134.5
	Skeletonema	7.83	30000	15	1	100	64.5	100	39.5
	costatum				2	100	62.5	100	35.5
					3	100	60.0	100	51.0
					4	100	111.8	100	45.5
					5	100	72.5	100	63.5
					6	100	75.0	100	67.5
					7	100	95.5	100	37.5

Larval Stage	Algal Species	Time Hours	Vial volume µL	Larval N	Vial Number	algal densities (cells µL^{-1}) control initial	control final	experimental initial	experimental final
NV	*Isochrysis*	7.58	30000	15	1	100	23.5	100	10.0
	galbana				2	100	53.4	100	19.1
					3	100	38.5	100	18.0
					4	100	40.0	100	21.0
					5	100	41.6	100	16.0
					6	100	46.2	100	16.5
					7	100	41.6	100	15.0
	Tetraselmis	7.1	30000	15	1	100	82.1	100	66.1
	chui				2	100	79.3	100	70.8
					3	100	80.7	100	74.2
					4	100	81.8	100	72.2
					5	100	76.5	100	62.9
					6	100	78.0	100	47.2
					7	100	71.8	100	69.4
	Skeletonema	7.6	30000	15	1	100	71.5	100	48.0
	costatum				2	100	68.5	100	42.5
					3	100	75.0	100	52.0
					4	100	117.8	100	52.5
					5	100	83.5	100	65.0
					6	100	82.0	100	53.0
					7	100	102.5	100	43.5

Larval Stage	Algal Species	Time Hours	Vial volume µL	Larval N	Vial Number	algal densities (cells µL^{-1})			
						control		experimental	
						initial	final	initial	final
NVI	*Isochrysis galbana*	7.3	30000	15	1	100	40.3	100	17.3
					2	100	60.5	100	25.5
					3	100	43.0	100	17.0
					4	100	41.0	100	21.0
					5	100	56.8	100	24.0
					6	100	52.3	100	21.5
					7	100	56.5	100	23.0
	Tetraselmis chui	6.97	30000	15	1	100	146.0	100	116.5
					2	100	91.8	100	82.9
					3	100	97.5	100	84.4
					4	100	82.5	100	70.6
					5	100	113.5	100	88.4
					6	100	106.8	100	86.6
					7	100	112.5	100	88.2
	Skeletonema costatum	7.75	30000	15	1	100	57.4	100	24.7
					2	100	73.5	100	38.5
					3	100	61.8	100	27.8
					4	100	65.7	100	31.0
					5	100	64.4	100	41.3
					6	100	65.1	100	56.3
					7	100	74.0	100	44.7

III.3 – Development and survival rates of *Pollicipes pollicipes* larvae under different algal food regimes (see section III.2 for algal diets composition)

day after hatching	algal diet	replicate	larval stages						
			NI	NII	NIII	NIV	NV	NVI	cyprid
3		1	0	22	132	0	0	0	0
	R	2	0	22	209	0	0	0	0
		3	0	33	161	0	0	0	0
		1	0	18	55	0	0	0	0
	S	2	0	18	169	0	0	0	0
		3	0	19	143	0	0	0	0
		1	0	18	172	0	0	0	0
	R or T	2	0	18	117	0	0	0	0
		3	0	22	114	0	0	0	0
		1	0	11	194	0	0	0	0
	R+S	2	0	22	128	0	0	0	0
		3	0	36	187	0	0	0	0
		1	0	12	176	0	0	0	0
	MIX	2	0	11	158	0	0	0	0
		3	0	11	150	0	0	0	0
6		1	0	0	19	73	0	0	0
	R	2	0	8	48	93	0	0	0
		3	0	0	24	85	0	0	0
		1	0	8	117	48	0	0	0
	S	2	0	0	129	12	0	0	0
		3	0	0	121	52	0	0	0
		1	0	0	32	73	0	0	0
	R or T	2	0	0	48	129	0	0	0
		3	0	12	16	73	0	0	0
		1	0	4	89	101	0	0	0
	R+S	2	0	0	81	109	0	0	0
		3	0	0	81	141	0	0	0
		1	0	4	36	149	4	0	0
	MIX	2	0	0	24	89	8	0	0
		3	0	8	61	105	0	0	0
9		1	0	0	10	19	76	10	0
	R	2	0	0	11	18	81	11	0
		3	0	0	0	11	62	22	0
		1	0	0	8	81	0	0	0
	S	2	0	0	54	50	0	0	0
		3	0	0	37	51	11	0	0
		1	0	0	0	8	117	8	0
	R or T	2	0	0	0	22	77	0	0
		3	0	0	0	15	92	0	0
		1	0	0	4	85	32	0	0
	R+S	2	0	0	12	48	4	0	0
		3	0	0	60	83	23	0	0
		1	0	0	0	31	70	9	0
	MIX	2	0	0	0	37	77	15	0
		3	0	0	0	37	51	4	0

day after hatching	algal diet	replicate	larval stages						
			NI	NII	NIII	NIV	NV	NVI	cyprid
12		1	0	0	0	4	52	44	0
	R	2	0	0	0	0	82	21	0
		3	0	0	0	0	40	93	0
		1	0	0	0	11	0	0	0
	S	2	0	0	26	15	0	0	0
		3	0	0	4	11	0	0	
		1	0	0	0	40	11	0	0
	R or T	2	0	0	4	59	22	0	0
		3	0	0	15	33	4	0	0
		1	0	0	0	40	4	0	0
	R+S	2	0	0	0	55	4	0	0
		3	0	0	0	36	4	0	0
		1	0	0	0	25	59	0	0
	MIX	2	0	0	4	55	15	0	0
		3	0	0	0	51	48	0	0
14		1	0	0	0	0	18	60	0
	R	2	0	0	0	0	23	82	3
		3	0	0	0	0	19	68	1
		1	0	0	0	0	4	4	0
	S	2	0	0	0	0	0	1	0
		3	0	0	0	0	0	0	0
		1	0	0	0	0	0	0	0
	R or T	2	0	0	0	0	1	0	0
		3	0	0	0	0	0	0	0
		1	0	0	0	0	0	0	0
	R+S	2	0	0	0	0	1	2	0
		3	0	0	0	0	1	0	0
		1	0	0	0	0	10	87	0
	MIX	2	0	0	0	0	9	82	0
		3	0	0	0	0	7	61	0

IV.1 – Larval measurements of *Elminius modestus* (µm), where larval stage 1 to 6 is nauplis I to VI and L is body total length, CSL carapace shield length, W greatest width, GL gut total length, and GW gut greatest width.

stage	L	CSL	W	GL	GW
1	336.6	*	122.8	*	*
1	357.0	*	133.0	*	*
1	387.6	*	143.2	*	*
1	387.6	*	153.4	*	*
1	387.6	*	143.2	*	*
1	357.0	*	133.0	*	*
1	367.2	*	143.2	*	*
1	408.0	*	143.2	*	*
1	377.4	*	133.0	*	*
1	397.8	*	133.0	*	*
1	408.0	*	143.2	*	*
1	367.2	*	133.0	*	*
1	377.4	*	143.2	*	*
1	408.0	*	133.0	*	*
1	224.4	*	82.0	*	*
2	387.6	*	142.8	122.4	71.4
2	357.0	*	153.0	132.6	71.4
2	387.6	*	153.0	132.6	51.0
2	397.8	*	173.4	142.8	61.2
2	428.4	*	153.0	142.8	61.2
2	397.8	*	142.8	132.6	51.0
2	397.8	*	142.8	122.4	61.2
2	387.6	*	122.4	122.4	61.2
2	397.8	*	153.0	102.0	51.0
2	397.8	*	153.0	122.4	61.2
2	377.4	*	*	112.2	61.2
3	408.0	*	234.6	102.0	51.0
2	367.2	*	122.4	102.0	51.0
2	408.0	*	122.4	122.4	61.2
2	408.0	*	163.2	102.0	40.8
2	408.0	*	*	132.6	*
2	418.2	*	142.8	132.6	51.0
2	408.0	*	*	132.6	*
2	428.4	*	142.8	142.8	61.2
2	397.8	*	142.8	122.4	51.0
2	387.6	*	142.8	132.6	51.0
2	377.4	*	142.8	142.8	30.6
2	397.8	*	153.0	132.4	51.0
2	408.0	*	173.4	112.2	61.2
2	367.2	*	132.6	112.2	51.0
2	428.4	*	153.0	122.4	51.0
3	450.0	*	200.0	130.0	*
3	450.0	*	200.0	100.0	60.0
3	440.0	*	200.0	100.0	60.0
3	420.0	*	190.0	110.0	70.0
3	420.0	*	200.0	130.0	40.0
3	440.0	*	210.0	140.0	70.0

stage	L	CSL	W	GL	GW
3	430.0	*	200.0	140.0	50.0
3	450.0	*	200.0	120.0	70.0
3	450.0	*	200.0	120.0	70.0
3	450.0	*	190.0	120.0	50.0
3	420.0	*	210.0	150.0	40.0
3	450.0	*	190.0	110.0	40.0
3	450.0	*	190.0	100.0	60.0
3	440.0	*	200.0	120.0	40.0
3	450.0	*	210.0	110.0	30.0
3	430.0	*	190.0	120.0	40.0
3	420.0	*	200.0	150.0	50.0
3	390.0	*	150.0	120.0	60.0
3	440.0	*	200.0	170.0	50.0
3	450.0	*	200.0	140.0	70.0
3	440.0	*	210.0	180.0	50.0
3	380.0	*	140.0	110.0	50.0
3	420.0	*	180.0	70.0	40.0
3	430.0	*	200.0	160.0	40.0
3	450.0	*	200.0	130.0	50.0
3	460.0	*	200.0	150.0	60.0
3	440.0	*	190.0	100.0	50.0
3	440.0	*	180.0	150.0	40.0
3	430.0	*	180.0	150.0	40.0
3	440.0	*	180.0	130.0	40.0
3	440.0	*	190.0	150.0	60.0
3	420.0	*	200.0	*	*
3	440.0	*	180.0	150.0	40.0
3	440.0	*	210.0	90.0	40.0
3	440.0	*	180.0	120.0	50.0
4	450.0	290.0	230.0	170.0	90.0
4	470.0	300.0	220.0	100.0	50.0
4	450.0	290.0	230.0	120.0	40.0
4	480.0	300.0	220.0	110.0	50.0
4	470.0	290.0	220.0	140.0	60.0
4	470.0	300.0	250.0	130.0	50.0
4	480.0	300.0	240.0	150.0	50.0
4	480.0	290.0	240.0	140.0	40.0
4	470.0	300.0	230.0	160.0	40.0
4	470.0	300.0	240.0	170.0	40.0
4	470.0	300.0	250.0	*	*
4	500.0	310.0	200.0	*	*
4	450.0	300.0	220.0	220.0	100.0
4	480.0	300.0	200.0	200.0	140.0
4	480.0	300.0	200.0	200.0	140.0
4	470.0	310.0	210.0	250.0	130.0
4	480.0	320.0	220.0	150.0	110.0
4	500.0	320.0	230.0	170.0	180.0
4	500.0	310.0	220.0	150.0	140.0
4	*	300.0	200.0	120.0	140.0
4	470.0	300.0	240.0	120.0	150.0
4	500.0	200.0	320.0	*	*
4	470.0	300.0	230.0	270.0	100.0
4	500.0	320.0	210.0	240.0	110.0

stage	L	CSL	W	GL	GW
4	490.0	320.0	230.0	200.0	100.0
4	470.0	300.0	230.0	210.0	100.0
4	470.0	300.0	270.0	160.0	160.0
4	450.0	290.0	220.0	200.0	140.0
4	480.0	280.0	230.0	250.0	130.0
4	490.0	310.0	230.0	150.0	110.0
4	460.0	300.0	230.0	170.0	180.0
4	450.0	330.0	250.0	150.0	140.0
4	480.0	300.0	220.0	120.0	140.0
4	450.0	290.0	220.0	120.0	120.0
4	480.0	280.0	230.0	130.0	*
4	490.0	300.0	220.0	230.0	100.0
4	460.0	300.0	230.0	240.0	110.0
4	450.0	330.0	250.0	200.0	100.0
4	480.0	300.0	220.0	*	*
4	*	*	250.0	*	*
4	450.0	300.0	250.0	240.0	150.0
4	400.0	310.0	250.0	250.0	70.0
4	470.0	290.0	200.0	180.0	100.0
5	530.0	350.0	220.0	120.0	100.0
5	560.0	360.0	300.0	*	*
5	530.0	360.0	310.0	*	*
5	540.0	330.0	290.0	280.0	150.0
5	520.0	280.0	330.0	280.0	130.0
5	530.0	330.0	280.0	230.0	120.0
5	520.0	350.0	240.0	290.0	100.0
5	540.0	360.0	300.0	290.0	130.0
5	540.0	360.0	280.0	280.0	130.0
5	480.0	340.0	230.0	290.0	120.0
5	540.0	360.0	260.0	280.0	130.0
5	520.0	350.0	240.0	280.0	150.0
5	540.0	360.0	300.0	270.0	160.0
5	540.0	360.0	280.0	290.0	110.0
5	520.0	340.0	290.0	*	*
5	480.0	340.0	230.0	280.0	130.0
5	540.0	380.0	260.0	290.0	120.0
5	520.0	340.0	*	270.0	160.0
5	520.0	340.0	*	260.0	130.0
5	550.0	370.0	270.0	*	*
5	530.0	380.0	*	*	*
5	500.0	380.0	*	290.0	110.0
5	460.0	340.0	280.0	280.0	130.0
5	480.0	330.0	*	290.0	120.0
5	550.0	380.0	270.0	*	*
5	510.0	320.0	250.0	230.0	120.0
5	470.0	*	180.0	*	*
5	510.0	330.0	240.0	290.0	100.0
5	500.0	320.0	230.0	280.0	130.0
5	380.0	*	180.0	*	*
5	490.0	300.0	230.0	290.0	120.0
5	430.0	310.0	220.0	290.0	130.0
5	510.0	320.0	250.0	*	*
5	510.0	330.0	240.0	180.0	170.0

stage	L	CSL	W	GL	GW
5	510.0	350.0	*	*	*
5	*	410.0	240.0	200.0	80.0
5	*	450.0	300.0	*	*
5	*	440.0	350.0	*	*
6	580.0	390.0	*	260.0	110.0
6	560.0	380.0	*	*	*
6	580.0	390.0	*	260.0	110.0
6	560.0	380.0	*	*	*
6	570.0	340.0	*	*	*
6	*	450.0	350.0	250.0	80.0
6	*	500.0	*	280.0	100.0
6	*	520.0	230.0	*	*
6	*	500.0	360.0	300.0	100.0
6	*	480.0	330.0	260.0	80.0
6	*	480.0	380.0	230.0	110.0
6	*	500.0	320.0	260.0	100.0
6	*	480.0	300.0	260.0	100.0
6	*	480.0	330.0	*	*
6	*	480.0	330.0	250.0	100.0
6	*	490.0	*	220.0	*
6	*	500.0	*	230.0	120.0
6	*	490.0	*	270.0	90.0
6	*	500.0	*	250.0	90.0
6	*	510.0	390.0	260.0	100.0
6	*	500.0	360.0	290.0	100.0
6	*	490.0	*	250.0	*
6	*	480.0	*	280.0	*
6	*	480.0	320.0	*	*
6	*	500.0	360.0	250.0	90.0
6	*	500.0	380.0	*	*
6	*	500.0	380.0	250.0	80.0
6	*	490.0	360.0	260.0	100.0
6	*	490.0	350.0	*	*
6	*	480.0	360.0	270.0	*
6	*	480.0	430.0	280.0	100.0
6	*	480.0	400.0	280.0	100.0
cyprid	*	550.0	200.0	*	*
cyprid	*	530.0	230.0	*	*
cyprid	*	520.0	220.0	*	*
cyprid	*	540.0	200.0	*	*
cyprid	*	550.0	210.0	*	*
cyprid	*	530.0	220.0	*	*
cyprid	*	530.0	220.0	*	*
cyprid	*	520.0	230.0	*	*
cyprid	*	540.0	200.0	*	*

IV.2 – Feeding rates of *Elminius modestus* NII on *Skeletonema costatum* and *Rhinomonas reticulata*

Time (Hours)	*R. reticulata* (cells µL⁻¹)				*S. costatum* (cells µL⁻¹)			
	control		experimental		control		Experimental	
	initial	final	Initial	final	initial	final	initial	final
24.48	179.17	192.50	177.08	100.83	0.00	0.00	0.00	0.00
24.22	156.25	142.92	150.83	87.92	37.08	29.58	24.58	17.92
24.08	137.50	136.67	124.58	104.58	67.92	65.00	77.08	61.67
24.00	115.83	102.08	117.92	61.67	107.08	120.42	84.58	86.67
23.87	94.58	102.50	99.58	88.33	134.58	121.11	130.42	81.67
23.75	77.92	69.58	85.83	75.42	165.83	174.17	168.75	165.00
23.65	63.75	53.75	57.50	47.50	249.17	217.92	203.33	157.92
23.57	45.00	35.42	43.33	30.00	269.58	220.42	228.33	143.75
23.47	0.00	0.00	0.00	0.00	265.00	262.92	284.58	220.00
23.35	332.92	372.08	332.92	353.75	0.00	0.00	0.00	0.00
23.38	295.42	365.42	278.75	305.83	46.67	33.33	60.42	20.83
23.50	197.92	267.08	262.08	281.25	81.25	61.25	100.83	50.00
23.63	184.58	194.58	183.33	124.17	110.00	81.25	94.17	40.83
23.77	179.17	232.50	182.50	177.08	149.58	123.33	152.08	106.25
24.02	112.08	137.08	145.00	130.83	244.58	154.17	215.00	107.50
24.15	88.33	111.67	88.33	85.83	258.33	201.67	261.67	151.67
24.28	43.33	56.25	51.67	56.25	350.42	293.75	318.33	211.25
24.42	0.00	0.00	0.00	0.00	425.83	399.58	456.67	311.25
25.72	246.67	304.17	257.08	271.25	0.00	0.00	0.00	0.00
25.65	191.67	245.42	253.75	217.08	26.25	38.33	37.08	15.83
25.60	198.75	208.33	196.67	200.00	58.75	70.83	69.58	37.92
25.57	151.25	160.83	189.58	190.42	81.67	104.58	100.00	76.25
25.52	142.08	164.17	152.08	135.00	130.83	120.83	116.25	100.00
25.45	102.08	127.08	99.58	80.83	166.25	155.83	170.00	76.25
25.35	76.67	85.42	80.00	77.08	193.33	160.83	182.50	142.92
25.30	44.58	51.25	41.67	41.25	217.08	267.92	237.50	170.83
25.25	0.00	0.00	0.00	0.00	249.58	288.33	253.75	232.08
24.97	422.08	376.67	415.83	370.83	0.00	0.00	0.00	0.00
24.93	295.83	358.33	322.08	364.58	132.08	120.42	129.17	67.92
24.90	232.50	276.67	250.42	252.08	236.67	213.33	294.17	174.58
24.88	237.50	216.67	261.67	210.21	468.75	312.92	395.83	212.92
24.87	206.25	255.00	195.83	211.25	459.17	377.08	475.42	257.50
24.85	163.75	221.67	219.58	196.67	639.58	368.33	832.50	274.17
24.83	109.17	93.33	118.75	90.83	802.50	360.83	820.83	332.50
24.80	77.50	62.50	76.67	56.67	893.33	598.33	885.83	381.25
24.78	0.00	0.00	0.00	0.00	1048.33	748.75	1284.17	585.83
24.03	286.67	322.08	330.00	339.17	0.00	0.00	0.00	0.00
23.92	289.17	290.00	286.67	260.83	41.67	48.33	49.58	31.25
23.83	199.17	240.00	225.83	224.17	93.75	92.08	97.08	65.00
23.73	182.50	185.00	179.58	164.58	120.00	162.50	135.83	110.42
23.67	146.25	163.33	143.75	127.08	171.67	216.25	181.25	162.50
23.57	116.67	125.83	121.25	120.00	222.08	244.17	194.58	204.58
23.47	81.25	85.42	74.58	74.17	240.83	289.17	246.67	212.50
23.37	44.17	42.50	50.83	38.33	306.67	361.67	340.83	291.67
23.30	0.00	0.00	0.00	0.00	369.17	408.33	359.17	355.00
25.72	246.67	304.17	257.08	271.25	0.00	0.00	0.00	0.00

Time (Hours)	*R. reticulata* (cells µL⁻¹)				*S. costatum* (cells µL⁻¹)			
	control		experimental		control		Experimental	
	initial	final	Initial	final	initial	final	initial	final
25.65	191.67	245.42	253.75	217.08	26.25	38.33	37.08	15.83
25.60	198.75	208.33	196.67	200.00	58.75	70.83	69.58	37.92
25.57	151.25	160.83	189.58	190.42	81.67	104.58	100.00	76.25
25.52	142.08	164.17	152.08	135.00	130.83	120.83	116.25	100.00
25.45	102.08	127.08	99.58	80.83	166.25	155.83	170.00	76.25
25.35	76.67	85.42	80.00	77.08	193.33	160.83	182.50	142.92
25.30	44.58	51.25	41.67	41.25	217.08	267.92	237.50	170.83
25.25	0.00	0.00	0.00	0.00	249.58	288.33	253.75	232.08

V.1 – *Pollicipes pollicipes* larval swimming speeds (nauplii IV, V, and VI)

observation	Nauplii IV speeds (mm sec^{-1})					
	Nauplii A	Nauplii B	Nauplii C	Nauplii D	Nauplii E	Nauplii F
1	1.14	1.34	1.12	1.65	1.84	1.15
2	1.00	0.90	0.96	1.37	1.21	1.03
3	0.88	1.18	0.90	1.03	0.84	0.79
4	0.72	0.79	0.79	0.95	0.56	0.58
5	•	0.71	•	0.88	0.43	•
6	•	•	•	0.70	•	•
7	•	•	•	0.54	•	•
8	•	•	•	0.51	•	•

Larval stage	Time (sec)	Distance (mm)	Speed (mm sec^{-1})
NIV	44.76	51	1.139
NIV	38.05	51	1.340
NIV	45.35	51	1.125
NIV	30.92	51	1.649
NIV	27.78	51	1.836
NIV	44.33	51	1.150
NV	30.27	51	1.685
NV	31.24	51	1.633
NV	29.02	51	1.757
NV	45.17	51	1.129
NV	49.08	51	1.039
NV	17.42	51	2.928
NV	24.31	51	2.098
NV	23.89	51	2.135
NV	12.47	51	4.090
NV	15.08	51	3.382
NV	34.25	51	1.489
NVI	14.16	51	3.602
NVI	12.21	51	4.177
NVI	25.4	51	2.008
NVI	42.01	51	1.214
NVI	18.88	51	2.701
NVI	23.92	51	2.132
NVI	14.99	51	3.402
NVI	9.03	51	5.648
NVI	17.66	51	2.888
NVI	7.91	51	6.448
NVI	48.74	51	1.046

V.2 – *Elminius modestus* larval swimming speeds (nauplii and cyprid)

Larval stage	Time (sec)	Distance (mm)	Speed (mm sec^{-1})
NI	1.21	1.41	1.17
NI	5.19	5.83	1.12
NI	3.39	5.00	1.47
NI	3.03	5.00	1.65
NI	1.30	2.83	2.18
NI	2.17	4.47	2.06
NI	9.28	10.00	1.08
NI	1.83	2.83	1.55
NI	2.54	5.83	2.30
NI	1.86	3.61	1.94
NI	2.44	5.83	2.39
NI	1.86	3.61	1.94
NI	2.44	2.83	1.16
NI	0.81	1.41	1.75
NI	2.05	3.61	1.76
NI	0.83	1.41	1.70
NI	3.23	3.00	0.93
NI	0.58	1.00	1.72
NI	1.39	2.00	1.44
NI	3.57	3.16	0.89
NI	0.77	1.41	1.84
NI	3.90	5.10	1.31
NI	0.61	1.41	2.32
NI	6.69	10.05	1.50
NI	5.25	8.06	1.54
NI	1.45	3.16	2.18
NI	3.32	5.39	1.62
NI	3.24	2.24	0.69
NI	2.42	3.00	1.24
NI	0.89	3.00	3.37
NI	0.47	1.00	2.13
NI	0.90	1.00	1.11
NI	0.48	1.00	2.08
NI	2.07	3.00	1.45
NI	1.92	3.16	1.65
NI	0.74	3.16	4.27
NI	0.64	1.00	1.56
NI	2.05	3.00	1.46
NI	3.19	4.12	1.29
NI	3.17	4.00	1.26
NI	0.89	1.41	1.59
NI	2.08	3.00	1.44
NI	9.01	10.00	1.11
NI	5.53	5.00	0.90
NI	3.52	4.00	1.14
NI	1.91	4.24	2.22
NI	1.48	2.00	1.35
NI	1.55	2.83	1.82
NI	2.05	4.16	2.03

larval stage	Time (sec)	Distance (mm)	Speed (mm sec^{-1})
NI	2.87	5.00	1.74
NI	0.37	1.00	2.70
NI	2.47	4.12	1.67
NI	4.96	6.00	1.21
NI	2.24	5.39	2.40
NI	2.79	6.00	2.15
NI	7.83	6.16	0.79
NI	3.13	5.00	1.60
NI	2.39	2.24	0.94
NI	3.17	6.61	2.08
NI	4.02	9.21	2.29
NI	1.53	3.16	2.07
NI	6.88	6.32	0.92
NI	0.72	1.41	1.96
NI	2.02	3.41	1.69
NI	1.92	3.61	1.88
NI	3.72	6.24	1.68
NI	8.25	10.05	1.22
NI	3.40	5.83	1.71
NI	1.53	2.00	1.31
NI	2.43	3.00	1.23
NI	9.28	11.10	1.20
NI	6.97	10.05	1.44
NI	7.37	8.47	1.15
NI	5.23	10.05	1.92
NI	5.14	6.08	1.18
NI	12.94	10.05	0.78
NI	4.53	3.16	0.70
NI	6.10	9.47	1.55
NI	5.47	7.07	1.29
NI	3.87	3.61	0.93
NI	3.37	3.16	0.94
NI	5.00	4.47	0.89
NII	0.80	2.24	2.80
NII	1.43	3.61	2.52
NII	0.30	1.00	3.33
NII	0.49	1.41	2.89
NII	0.77	2.00	2.60
NII	1.11	3.16	2.85
NII	0.87	2.24	2.57
NII	1.09	3.61	3.31
NII	0.86	2.41	2.81
NII	1.81	7.07	3.91
NII	0.67	3.00	4.48
NII	1.42	4.00	2.82
NII	0.87	3.16	3.63
NII	1.93	7.16	3.71
NII	2.50	6.12	2.45
NII	1.53	4.83	3.16

Larval stage	Time (sec)	Distance (mm)	Speed (mm sec^{-1})
NII	2.53	7.07	2.79
NII	2.59	5.39	2.08
NII	2.90	8.40	2.90
NII	1.32	3.00	2.27
NII	3.15	9.00	2.86
NII	2.45	3.61	1.47
NII	0.87	1.41	1.63
NII	2.38	3.16	1.33
NII	4.42	10.39	2.35
NII	1.97	4.00	2.03
NII	3.75	6.71	1.79
NII	5.37	10.20	1.90
NII	7.94	11.40	1.44
NII	8.64	9.49	1.10
NII	8.20	8.06	0.98
NII	7.34	11.05	1.50
NII	9.50	8.94	0.94
NII	4.63	5.00	1.08
NII	0.98	4.00	4.08
NII	7.11	5.00	0.70
NII	2.41	7.39	3.06
NII	2.50	5.83	2.33
NII	6.80	12.00	1.76
NII	4.35	9.49	2.18
NII	19.29	10.44	0.54
NII	3.27	9.06	2.77
NII	3.85	11.05	2.87
NII	5.00	10.00	2.00
NII	4.56	9.49	2.08
NII	3.88	10.00	2.58
NII	1.67	5.00	2.99
NII	5.45	10.00	1.83
NII	4.00	10.20	2.55
NII	3.50	5.00	1.43
NII	6.31	7.28	1.15
NII	2.76	11.40	4.13
NII	1.24	3.00	2.42
NII	2.31	3.61	1.56
NII	5.49	11.18	2.04
NII	4.94	4.12	0.83
NII	4.88	6.24	1.28
NII	6.35	8.25	1.30
NII	1.50	3.00	2.00
NII	5.26	9.00	1.71
NII	3.89	6.32	1.63
NII	7.20	6.16	0.86
NII	2.47	2.00	0.81
NII	4.63	6.08	1.31
NII	4.98	4.00	0.80
NII	3.03	12.00	3.96
NII	2.41	10.39	4.31
NII	2.60	7.00	2.69

Larval stage	Time (sec)	Distance (mm)	Speed (mm sec^{-1})
NII	4.71	5.10	1.08
NII	5.06	9.85	1.95
NII	4.04	7.07	1.75
NII	1.15	8.25	7.17
NII	1.68	5.83	3.47
NII	3.49	5.00	1.43
NII	2.91	5.00	1.72
NII	5.00	6.00	1.20
NII	2.73	8.06	2.95
NII	3.46	7.00	2.02
NII	2.90	5.10	1.76
NII	1.53	8.54	5.58
NII	2.50	7.07	2.83
NII	3.75	10.00	2.67
NII	3.72	10.00	2.69
NII	5.07	10.20	2.01
NII	4.85	10.00	2.06
NII	3.38	11.05	3.27
NII	2.11	6.00	2.84
NII	2.53	10.05	3.97
NII	1.75	8.06	4.61
NII	4.67	12.73	2.73
NII	1.42	6.08	4.28
NII	2.58	6.32	2.45
NII	5.50	10.00	1.82
NII	2.09	6.32	3.03
NII	1.20	3.16	2.64
NIII	3.67	6.32	1.72
NIII	3.46	6.08	1.76
NIII	2.38	7.00	2.94
NIII	1.00	5.39	5.39
NIII	3.67	10.00	2.72
NIII	2.23	6.71	3.01
NIII	2.05	19.44	9.48
NIII	2.00	6.00	3.00
NIII	3.39	1.41	0.42
NIII	3.52	9.06	2.57
NIII	2.80	8.06	2.88
NIII	2.90	9.22	3.18
NIII	3.96	9.85	2.49
NIII	1.93	10.05	5.21
NIII	4.12	10.00	2.43
NIII	3.10	8.94	2.89
NIII	2.78	8.39	3.02
NIII	4.66	8.25	1.77
NIII	1.41	5.00	3.55
NIII	3.22	11.00	3.42
NIII	3.00	10.05	3.35
NIII	0.64	4.12	6.44
NIII	1.51	11.22	7.43
NIII	1.78	9.22	5.18
NIII	1.55	5.00	3.23

Larval stage	Time (sec)	Distance (mm)	Speed (mm sec^{-1})
NIII	0.81	3.00	3.70
NIII	1.01	5.00	4.95
NIII	2.00	8.25	4.12
NIII	1.88	7.00	3.72
NIII	2.06	9.22	4.48
NIII	2.89	10.00	3.46
NIII	4.35	9.32	2.14
NIII	1.61	6.32	3.93
NIII	1.31	8.00	6.11
NIII	2.47	10.16	4.11
NIII	2.69	11.71	4.35
NIII	1.09	6.08	5.58
NIII	2.56	9.36	3.66
NIII	1.32	7.07	5.36
NIII	2.15	8.49	3.95
NIII	1.66	11.00	6.63
NIII	2.95	11.18	3.79
NIII	1.05	3.16	3.01
NIII	2.53	11.05	4.37
NIII	1.36	5.00	3.68
NIII	1.45	6.71	4.63
NIII	1.41	5.00	3.55
NIII	0.96	5.10	5.31
NIII	2.52	8.54	3.39
NIII	1.22	6.00	4.92
NIII	0.43	2.24	5.20
NIII	1.69	7.62	4.51
NIII	0.72	3.16	4.39
NIII	0.83	4.00	4.82
NIII	1.89	10.34	5.47
NIII	1.62	9.49	5.86
NIII	0.88	5.00	5.68
NIII	0.91	4.12	4.53
NIII	0.93	5.00	5.38
NIII	1.33	8.25	6.20
NIII	1.52	9.06	5.96
NIII	0.70	4.12	5.89
NIII	1.71	7.29	4.26
NIII	1.63	9.00	5.52
NIII	1.72	6.71	3.90
NIII	0.89	5.10	5.73
NIII	1.51	6.00	3.97
NIII	0.95	5.39	5.67
NIII	1.30	6.71	5.16
NIII	1.72	11.00	6.40
NIII	1.26	6.08	4.83
NIII	0.84	4.12	4.91
NIII	1.44	5.39	3.74
NIII	1.27	5.00	3.94
NIV	2.72	5.83	2.14
NIV	0.69	3.16	4.58
NIV	1.15	3.16	2.75
NIV	1.07	6.08	5.68
NIV	3.19	7.21	2.26
NIV	0.91	3.16	3.48
NIV	1.58	8.06	5.10
NIV	1.69	6.71	3.97
NIV	2.06	8.25	4.00
NIV	2.36	9.10	3.86
NIV	1.41	5.00	3.55
NIV	2.01	8.06	4.01
NIV	1.85	5.10	2.76
NIV	1.53	9.00	5.88
NIV	0.93	5.39	5.79
NIV	0.73	5.00	6.85
NIV	0.40	7.28	18.20
NIV	0.99	4.00	4.04
NIV	1.21	7.41	6.13
NIV	1.01	9.49	9.39
NIV	0.44	3.16	7.19
NIV	1.13	7.07	6.26
NIV	1.18	7.00	5.93
NIV	2.29	10.05	4.39
NIV	1.21	8.25	6.82
NIV	1.00	7.00	7.00
NIV	2.39	11.18	4.68
NIV	1.73	10.00	5.78
NIV	1.04	5.39	5.18
NIV	2.46	10.47	4.26
NIV	2.23	9.22	4.13
NIV	0.58	3.16	5.45
NIV	0.61	3.00	4.92
NIV	1.96	4.12	2.10
NIV	0.67	5.10	7.61
NIV	1.43	7.62	5.33
NIV	1.14	7.62	6.68
NIV	1.14	6.71	5.88
NIV	2.68	8.94	3.34
NIV	0.85	5.83	6.86
NIV	1.60	6.00	3.75
NIV	1.72	10.00	5.81
NIV	7.14	7.28	1.02
NIV	1.68	10.32	6.15
NIV	1.71	10.20	5.96
NIV	1.03	3.61	3.50
NIV	2.00	9.43	4.72
NIV	1.52	10.00	6.58
NIV	1.78	6.32	3.55
NIV	1.09	4.47	4.10
NIV	1.39	5.39	3.87
NIV	1.25	5.39	4.31
NV	2.14	5.39	2.52
NV	0.67	3.61	5.38
NV	0.69	4.24	6.15
NV	3.42	11.91	3.48
NV	2.82	10.05	3.56

Larval stage	Time (sec)	Distance (mm)	Speed (mm sec^{-1})
NV	1.22	4.12	3.38
NV	1.36	4.47	3.29
NV	1.47	5.39	3.66
NV	1.20	5.00	4.17
NV	1.72	6.08	3.54
NV	1.46	6.00	4.11
NV	1.23	6.08	4.95
NV	1.51	6.08	4.03
NV	0.76	4.12	5.43
NV	2.13	6.00	2.82
NV	1.69	6.00	3.55
NV	1.77	6.08	3.44
NV	1.98	6.08	3.07
NV	2.06	7.00	3.40
NV	0.98	8.00	8.16
NV	1.16	9.06	7.81
NV	1.08	3.61	3.34
NV	0.69	3.00	4.35
NV	0.91	9.06	9.95
NV	0.69	6.40	9.28
NV	1.20	6.32	5.27
NV	1.16	8.49	7.31
NV	0.40	3.61	9.01
NV	0.88	11.08	12.59
NV	0.88	9.43	10.72
NV	1.54	11.00	7.14
NV	1.29	9.06	7.02
NV	0.83	5.00	6.02
NV	1.30	7.62	5.86
NV	1.44	11.00	7.64
NV	1.08	11.05	10.23
NV	0.88	7.00	7.95
NV	0.87	5.10	5.86
NV	0.71	4.12	5.81
NV	1.57	6.32	4.03
NV	1.20	8.25	6.87
NV	0.76	5.00	6.58
NV	1.05	5.39	5.13
NV	0.83	3.61	4.34
NV	1.34	10.32	7.70
NV	2.15	9.06	4.21
NV	2.48	5.39	2.17
NV	1.43	10.00	6.99
NV	0.89	5.10	5.73
NV	1.20	8.54	7.12
NV	1.53	10.83	7.08
NV	2.10	10.77	5.13
NV	0.87	5.39	6.19
NV	1.38	9.95	7.21
NV	1.45	10.00	6.90
NV	0.54	3.16	5.86
NV	1.03	3.61	3.50
NV	0.88	5.39	6.12

Larval stage	Time (sec)	Distance (mm)	Speed (mm sec^{-1})
NV	1.00	3.61	3.61
NV	0.51	3.61	7.07
NV	1.03	7.00	6.80
NV	1.16	5.83	5.03
NV	1.24	6.32	5.10
NV	1.35	9.06	6.71
NV	1.25	6.00	4.80
NV	1.50	5.10	3.40
NV	0.54	4.00	7.41
NV	1.00	6.08	6.08
NV	0.43	3.16	7.35
NV	1.42	9.00	6.34
NV	1.08	8.00	7.41
NV	1.32	9.06	6.86
NV	1.50	10.00	6.67
NV	1.20	7.28	6.07
NV	1.37	7.07	5.16
NV	1.03	8.06	7.83
NV	1.32	9.22	6.98
NV	1.53	11.00	7.19
NV	2.48	9.47	3.82
NV	1.59	8.00	5.03
NV	1.14	7.21	6.33
NV	1.92	11.05	5.75
NV	1.99	11.70	5.88
NV	1.60	9.22	5.76
NVI	1.38	9.00	6.52
NVI	0.94	5.66	6.02
NVI	1.64	11.08	6.76
NVI	2.65	6.40	2.42
NVI	3.66	6.32	1.73
NVI	1.52	10.20	6.71
NVI	2.73	11.19	4.10
NVI	1.96	7.62	3.89
NVI	2.08	9.00	4.33
NVI	1.39	8.06	5.80
NVI	2.90	9.06	3.12
NVI	2.42	8.10	3.35
NVI	4.14	10.20	2.46
NVI	1.43	10.00	6.99
NVI	1.20	11.18	9.32
NVI	4.10	11.66	2.84
NVI	3.11	8.94	2.88
NVI	2.14	8.06	3.77
NVI	2.32	10.05	4.33
NVI	2.22	7.62	3.43
NVI	1.45	7.28	5.02
NVI	1.12	7.07	6.31
NVI	2.04	11.00	5.39
NVI	1.36	4.00	2.94
NVI	0.81	5.39	6.65
NVI	4.42	12.53	2.83
NVI	1.99	8.60	4.32

Larval stage	Time (sec)	Distance (mm)	Speed (mm sec^{-1})
NVI	1.22	10.48	8.59
NVI	0.96	5.66	5.89
NVI	1.27	5.10	4.01
NVI	1.56	9.39	6.02
NVI	1.31	6.08	4.64
NVI	2.36	10.20	4.32
NVI	2.20	9.12	4.15
NVI	1.57	9.49	6.04
NVI	1.08	7.62	7.05
NVI	1.61	11.94	7.42
NVI	1.74	11.05	6.35
NVI	1.55	11.00	7.10
NVI	1.23	9.66	7.85
NVI	2.34	16.37	6.99
NVI	1.62	8.94	5.52
NVI	1.48	4.12	2.79
NVI	1.61	9.85	6.12
NVI	1.64	10.20	6.22
NVI	1.46	8.25	5.65
NVI	1.26	8.25	6.54

Larval stage	Time (sec)	Distance (mm)	Speed (mm sec^{-1})
cyprid	2.26	7.81	3.46
cyprid	1.07	11.67	10.91
cyprid	3.32	5.83	1.76
cyprid	3.17	7.47	2.36
cyprid	2.61	22.59	8.66
cyprid	0.78	12.00	15.38
cyprid	2.51	12.48	4.97
cyprid	0.77	4.47	5.81
cyprid	0.78	6.32	8.11
cyprid	2.58	5.83	2.26
cyprid	1.72	7.83	4.55
cyprid	0.72	11.40	15.84
cyprid	0.57	5.39	9.45
cyprid	1.39	8.26	5.94
cyprid	0.67	6.71	10.01
cyprid	0.20	12.81	64.03
cyprid	0.64	11.40	17.82
cyprid	1.41	10.30	7.30
cyprid	0.82	5.00	6.10

V.3 – *Elminius modestus* larval swimming speeds (response to food type – NVI larvae)

Rhinomonas reticulata		
Time (sec)	Distance (mm)	Speed (mm sec^{-1})
1.38	9.00	6.52174
0.94	5.6569	6.01793
1.64	11.0828	6.75778
2.65	6.4031	2.41627
3.66	6.3246	1.72802
1.52	10.198	6.70924
2.73	11.1942	4.10043
1.96	7.6158	3.8856
2.08	9.00	4.32692
1.39	8.0623	5.80019
2.9	9.0554	3.12255
2.42	8.099	3.3467
4.14	10.198	2.46329
1.43	10.00	6.99301
1.2	11.1803	9.31695
4.1	11.6619	2.84437
3.11	8.9443	2.87597
2.14	8.0623	3.76741
2.32	10.0499	4.33184
2.22	7.6158	3.43053
1.45	7.2801	5.02077
1.12	7.0711	6.31345
2.04	11.0	5.39216
1.36	4.00	2.94118
0.81	5.3852	6.64835
4.42	12.53	2.83483
1.99	8.5952	4.31922
1.99	8.2462	4.14382
1.22	10.4842	8.59359
0.96	5.6569	5.89256
1.27	5.099	4.01498
1.56	9.3852	6.01613
1.31	6.0828	4.64333
2.36	10.198	4.3212
2.2	9.1231	4.14687
1.57	9.4868	6.04257
1.08	7.6158	7.05164
1.61	11.9443	7.4188
1.74	11.0454	6.34791
1.55	11.00	7.09677
1.23	9.6604	7.854
2.34	16.3657	6.99391
1.62	8.9443	5.52116
1.48	4.1231	2.78588
1.61	9.8489	6.1173
1.64	10.198	6.21832
1.46	8.2462	5.64809
1.26	8.2462	6.54461
1.95	9.0554	4.64379

Mixed algal culture		
Time (sec)	Distance (mm)	Speed (mm sec^{-1})
3.5	9.2195	2.6342
2.47	6.00	2.4291
2.19	6.0828	2.7775
1.7	4.1231	2.4254
2.71	7.2801	2.6864
2.23	5.6569	2.5367
1.15	4.4721	3.8888
1.14	5.00	4.386
3.92	8.0623	2.0567
1.94	6.00	3.0928
1.52	5.099	3.3546
1.28	6.0828	4.7522
3.15	5.831	1.8511
2.24	11.1623	4.9832
0.75	7.2801	9.7068
0.86	8.831	10.2685
1.39	11.4018	8.2027
1.76	9.434	5.3602
0.73	7.2801	9.9728
1.08	8.0623	7.4651
1.05	8.2462	7.8535
1.83	10.0499	5.4917
1.69	6.00	3.5503

V.4 – *Elminius modestus* larval swimming speeds (Response to starvation - NII larvae)

Starving NII larvae			
Time of experiment (minutes)	Time (sec)	Distance (mm)	Speed (mm sec)
0	0.36	4.24	11.79
0	2.07	5.10	2.46
0	1.07	8.94	8.36
0	1.05	7.62	7.25
0	1.43	9.49	6.63
0	1.30	10.20	7.84
0	5.15	10.00	1.94
0	1.85	8.54	4.62
0	3.23	7.07	2.19
0	3.09	6.08	1.97
0	1.50	3.61	2.40
27	1.60	6.40	4.00
27	2.53	8.25	3.26
27	3.83	10.00	2.61
27	1.59	11.18	7.03
86	3.32	9.22	2.78
86	2.02	8.06	3.99
86	2.20	6.71	3.05
86	1.51	5.83	3.86
86	4.19	9.49	2.26
86	1.09	5.00	4.59
86	1.64	9.85	6.01
86	2.29	6.32	2.76
86	1.89	9.00	4.76
86	1.55	5.10	3.29
86	1.23	11.00	8.94
159	3.38	9.06	2.68
159	1.20	9.43	7.86
159	2.06	8.00	3.88
160	1.74	5.39	3.09
160	1.23	6.00	4.88
160	1.96	7.00	3.57
160	2.07	5.39	2.60
160	1.35	7.07	5.24
160	2.38	9.00	3.78
160	2.17	7.00	3.23
160	3.93	9.06	2.30
160	1.03	5.39	5.23
160	2.99	9.22	3.08
160	1.65	5.83	3.53
163	1.67	7.62	4.56
163	2.29	8.25	3.60
163	3.01	8.25	2.74
163	2.57	7.62	2.96
163	2.45	7.07	2.89

Starving NII larvae			
Time of experiment (minutes)	Time (sec)	Distance (mm)	Speed (mm sec)
163	2.08	8.54	4.11
163	1.69	10.00	5.92
163	2.76	9.49	3.44
163	3.23	7.00	2.17
163	3.23	10.20	3.16
163	3.33	9.49	2.85
206	0.87	5.10	5.86
206	1.11	8.06	7.26
206	2.14	7.07	3.30
206	0.99	7.07	7.14
263	2.64	7.07	2.68
263	1.66	8.00	4.82
263	1.54	7.00	4.55
263	2.13	7.28	3.42
263	1.92	7.62	3.97
263	2.29	9.06	3.95
265	1.69	7.00	4.14
265	1.90	5.00	2.63
265	1.38	7.00	5.07
265	2.06	11.00	5.34
265	2.10	10.00	4.76
265	2.25	10.05	4.47
265	2.33	10.05	4.31
265	4.28	5.10	1.19
265	1.78	7.07	3.97
265	1.75	7.28	4.16
265	1.66	10.00	6.02
268	1.16	9.49	8.18
268	2.86	8.54	2.99
268	1.35	10.05	7.44
268	1.82	11.05	6.07
268	1.39	7.28	5.24
268	3.15	11.18	3.55
268	3.29	8.54	2.60
268	1.41	8.00	5.67
268	1.49	8.25	5.53
268	1.26	7.07	5.61
268	4.53	11.31	2.50
352	2.29	10.77	4.70
352	2.48	8.25	3.33
352	2.25	6.71	2.98
352	2.89	8.60	2.98
352	5.17	9.22	1.78
352	2.89	8.25	2.85
353	2.97	9.06	3.05

Starving NII larvae			
Time of experiment (minutes)	Time (sec)	Distance (mm)	Speed (mm sec-)
353	1.30	4.00	3.08
353	4.32	8.54	1.98
353	1.17	5.39	4.60
353	1.22	7.00	5.74
353	2.87	8.00	2.79
353	1.36	6.32	4.65
353	1.67	5.00	2.99
353	1.42	9.06	6.38
353	1.33	10.20	7.67
353	1.62	11.40	7.04
357	2.02	8.25	4.08
357	2.93	7.00	2.39
357	0.80	6.08	7.60
357	1.57	10.05	6.40
357	1.73	7.62	4.40
357	2.92	9.49	3.25
358	4.30	8.60	2.00
358	1.69	9.85	5.83
358	2.24	5.00	2.23
358	1.89	8.00	4.23
358	2.58	6.00	2.33
358	2.21	11.05	5.00
358	3.73	9.85	2.64
358	2.89	10.05	3.48
358	4.27	10.05	2.35
359	1.60	10.05	6.28
359	2.33	9.49	4.07
359	1.80	7.28	4.04
359	1.66	5.66	3.41
359	1.73	4.12	2.38
359	1.64	4.12	2.51
359	0.91	3.16	3.48
359	1.77	3.61	2.04
359	1.44	3.00	2.08
359	1.50	3.16	2.11
359	3.50	7.07	2.02
359	1.78	6.71	3.77
420	2.17	5.39	2.48
420	1.46	9.61	6.58
420	0.77	5.39	6.99
420	3.25	8.00	2.46
420	1.00	5.83	5.83
423	1.50	3.61	2.40
423	1.91	5.83	3.05
423	3.06	11.40	3.73
423	1.12	5.10	4.55
423	1.37	5.39	3.93
423	2.17	9.22	4.25

Starving NII larvae			
Time of experiment (minutes)	Time (sec)	Distance (mm)	Speed (mm sec-)
481	2.95	10.20	3.46
481	2.10	10.20	4.86
481	2.08	9.16	4.40
481	1.90	8.06	4.24
481	3.78	11.40	3.02
481	0.81	5.39	6.65
485	1.41	5.00	3.55
485	5.42	11.18	2.06
485	1.39	7.07	5.09
485	3.37	8.25	2.45
485	1.55	7.00	4.52
539	1.67	7.07	4.23
539	1.87	6.32	3.38
539	1.61	10.20	6.33
539	0.97	5.10	5.26
539	1.74	7.62	4.38
539	2.69	10.00	3.72
539	1.75	6.71	3.83
539	2.67	11.18	4.19
539	2.83	10.77	3.81
539	2.32	7.00	3.02
539	1.67	8.06	4.83
598	4.72	11.00	2.33
598	2.20	9.39	4.27
598	2.12	10.82	5.10
598	1.35	5.83	4.32
598	1.77	10.63	6.01
598	1.68	6.71	3.99
598	2.19	6.32	2.89
598	2.53	11.41	4.51
598	1.51	7.28	4.82
1198	1.99	6.71	3.37
1198	2.06	10.44	5.07
1202	1.03	5.00	4.85
1202	1.71	5.00	2.92
1202	2.27	5.39	2.37
1202	0.45	2.83	6.29
1202	1.62	10.24	6.32
1202	2.73	10.05	3.68
1202	2.98	8.06	2.71
1204	2.23	10.24	4.59
1204	1.50	10.16	6.77
1204	2.50	10.00	4.00
1204	1.62	10.05	6.20
1204	2.18	11.05	5.07
1204	1.88	9.22	4.90
1269	1.39	6.32	4.55
1269	2.34	10.10	4.32

Starving NII larvae			
Time of experiment (minutes)	Time (sec)	Distance (mm)	Speed (mm sec)
1269	1.04	5.39	5.18
1269	4.03	7.81	1.94
1269	1.02	5.00	4.90
1269	0.70	4.47	6.39
1269	1.34	8.06	6.02
1275	1.35	7.81	5.79
1275	1.06	7.62	7.18
1275	1.23	7.07	5.75
1275	1.25	7.07	5.66
1339	1.50	10.05	6.70
1339	0.69	4.12	5.98
1339	1.43	8.16	5.71
1339	2.02	10.05	4.98
1339	1.92	7.28	3.79
1339	2.42	7.28	3.01
1339	2.35	9.78	4.16
1344	0.90	4.00	4.44
1344	1.75	8.00	4.57
1344	0.76	4.47	5.88
1344	3.79	12.04	3.18
1344	1.60	6.00	3.75
1344	3.57	10.44	2.92
1407	2.69	8.00	2.97
1407	3.72	10.00	2.69
1407	1.04	5.39	5.18
1407	1.45	7.07	4.88
1407	2.09	10.12	4.84
1407	2.77	10.00	3.61
1407	2.68	10.40	3.88
1407	2.44	10.00	4.10
1471	1.17	7.00	5.98
1471	2.72	9.06	3.33
1471	2.06	6.77	3.29
1471	3.92	10.12	2.58
1471	1.03	4.47	4.34
1471	3.07	10.05	3.27
1471	3.41	9.39	2.75
1537	1.51	9.00	5.96
1537	1.38	8.25	5.98
1537	2.79	10.21	3.66
1537	1.67	6.16	3.69
1537	1.96	9.10	4.64

Starving NII larvae			
Time of experiment (minutes)	Time (sec)	Distance (mm)	Speed (mm sec)
1537	2.03	9.00	4.43
1537	1.16	5.10	4.40
1593	2.99	10.20	3.41
1593	1.77	6.71	3.79
1593	2.75	9.10	3.31
1593	1.96	11.32	5.78
1593	2.90	10.10	3.48
1593	2.03	10.10	4.97
1593	2.78	11.00	3.96
1594	1.80	9.49	5.27
1594	3.03	10.44	3.45
1594	2.06	8.25	4.00
1594	2.28	7.62	3.34
1594	1.84	6.32	3.44
1596	2.29	9.22	4.03
1596	1.93	8.25	4.27
1596	1.68	7.28	4.33
1597	2.63	8.00	3.04
1597	1.54	5.83	3.79
1597	4.63	11.00	2.38
1597	5.11	10.00	1.96
1597	3.35	11.47	3.42
1597	1.98	12.08	6.10
1681	2.54	6.71	2.64
1681	2.20	11.00	5.00
1681	2.94	12.00	4.08
1681	2.10	9.00	4.29
1681	3.69	11.91	3.23
1681	1.89	7.21	3.82
1681	1.25	6.32	5.06
1681	2.33	10.00	4.29
1681	2.70	11.24	4.16
1681	2.88	11.05	3.84
1685	2.78	8.62	3.10
1685	3.81	10.62	2.79
1685	1.59	4.00	2.52
1685	3.57	9.00	2.52
1687	3.60	10.45	2.90
1687	2.22	8.12	3.66
1687	3.47	10.08	2.91
1687	3.65	11.39	3.12

Feeding NII larvae			
Time of experiment (minutes)	Time (sec)	Distance (mm)	Speed (mm sec)
10	2.63	4.00	1.52
10	1.44	6.32	4.39
10	2.60	6.71	2.58
10	4.07	9.06	2.22
10	1.41	8.00	5.67
10	1.25	10.00	8.00
10	1.29	8.25	6.39
10	1.33	7.00	5.26
10	0.92	5.83	6.34
14	1.91	8.94	4.68
14	1.42	5.83	4.11
14	1.05	6.71	6.39
14	2.07	6.32	3.06
14	2.40	6.32	2.64
14	1.41	6.71	4.76
14	4.05	11.39	2.81
14	1.80	7.00	3.89
14	3.83	9.24	2.41
14	1.30	11.18	8.60
14	2.33	4.00	1.72
20	3.76	7.00	1.86
20	4.07	8.06	1.98
20	3.82	8.06	2.11
20	2.33	4.00	1.72
20	2.33	4.00	1.72
20	1.13	5.39	4.77
20	0.84	5.66	6.73
20	1.78	11.00	6.18
20	2.23	10.44	4.68
20	1.28	8.00	6.25
209	2.25	6.24	2.77
209	3.23	8.25	2.55
209	1.15	8.25	7.17
209	2.51	8.60	3.43
209	1.18	10.77	9.13
209	1.51	8.49	5.62
209	1.27	8.25	6.49
209	0.97	8.60	8.87
209	1.50	8.00	5.33
209	1.23	7.28	5.92
209	1.13	6.32	5.60
361	1.50	10.05	6.70
361	1.66	10.20	6.14
361	2.11	8.25	3.91
361	1.84	6.08	3.31

Feeding NII larvae			
Time of experiment (minutes)	Time (sec)	Distance (mm)	Speed (mm sec)
361	0.99	5.39	5.44
361	2.09	9.22	4.41
361	2.13	6.32	2.97
361	2.69	9.06	3.37
361	1.21	10.05	8.31
361	1.59	11.18	7.03
361	2.16	5.83	2.70
365	5.09	10.44	2.05
365	5.13	7.28	1.42
365	3.50	10.44	2.98
365	1.67	7.00	4.19
365	1.53	7.00	4.58
627	1.75	10.00	5.71
627	1.54	10.20	6.62
627	1.12	10.44	9.32
627	1.92	8.06	4.20
627	2.01	10.00	4.98
627	1.03	8.25	8.01
627	1.83	10.05	5.49
627	1.77	10.05	5.68
627	1.13	9.00	7.96
627	1.23	9.06	7.36
1200	1.63	8.06	4.95
1200	2.62	10.05	3.84
1200	1.60	9.61	6.00
1200	2.04	9.22	4.52
1200	1.01	8.94	8.86
1200	0.98	6.32	6.45
1200	1.83	11.05	6.04
1200	4.20	6.32	1.51
1200	1.97	8.06	4.09
1405	1.40	6.32	4.52
1405	1.20	5.39	4.49
1405	1.50	8.00	5.33
1405	2.01	8.94	4.45
1405	1.57	8.25	5.25
1405	1.73	10.77	6.23
1405	1.56	8.60	5.51
1405	1.21	10.05	8.31
1644	3.02	7.28	2.41
1644	0.79	5.66	7.16
1644	1.27	6.00	4.72
1644	2.62	10.44	3.98
1644	0.84	7.21	8.58

VI.1 – Dry Weight of *Elminius modestus* nauplii.

stage	aluminium boats (mg)	w/ larvae (mg)	300 larvae (mg)	1 larvae (µg)
NI	14.100	14.258	0.158	0.527
NI	13.689	13.853	0.164	0.547
NI	14.439	14.586	0.147	0.490
NII	15.040	15.283	0.243	0.810
NII	15.973	16.195	0.222	0.740
NII	14.937	15.148	0.211	0.703
NIII	15.015	15.358	0.343	1.143
NIII	14.633	14.954	0.321	1.070
NIII	14.403	14.735	0.332	1.107
NIV	15.079	15.677	0.598	1.993
NIV	15.582	16.149	0.567	1.890
NIV	15.381	15.924	0.543	1.810
NV	16.709	17.596	0.887	2.957
NV	14.160	15.074	0.914	3.047
NV	15.296	16.196	0.900	3.000
NVI	15.602	17.3	1.698	5.660
NVI	15.069	16.711	1.642	5.473
NVI	15.069	16.668	1.599	5.330

VI.2 – Oxygen consumption of newly hatched *Elminius modestus* larvae

Temperature (°C)	experimental time (minutes)	solution	chamber volume (µL)	Larval N	Oxygen drop slope	Max [O2] in chamber (µL Oxigen)	Respiration (nL O2 animal^{-1}hour^{-1})
17.87	68.29	sea H2O	150	203	10.53684	0.83415	2.45368
17.87	71.35	sea H2O	180	160	12.19112	1.00098	4.28766
17.88	154.96	sea H2O	150	100	6.92218	0.83399	3.28151
17.88	159.23	sea H2O	180	70	5.60983	1.00078	4.35192
17.88	232.85	sea H2O	150	156	6.86786	0.83399	2.09271
17.88	237.25	sea H2O	180	86	4.37527	1.00078	2.67991
17.89	298.71	sea H2O	150	138	7.65030	0.83383	2.64076
17.89	303.58	sea H2O	180	71	2.29527	1.00059	1.66027
17.9	363.10	sea H2O	150	64	2.30113	0.83367	1.71629
17.9	365.50	sea H2O	180	99	2.24839	1.00040	1.13972
17.9	427.07	sea H2O	150	161	3.92150	0.83367	1.16528
17.9	431.94	sea H2O	180	100	1.68728	1.00040	0.82663
16.88	558.96	sea H2O	150	52	1.73643	0.85024	0.93106
16.88	560.03	sea H2O	180	86	0.35064	1.02028	0.13169
16.9	621.31	sea H2O	150	199	0.80089	0.84990	0.11406
16.9	622.31	sea H2O	180	34	0.46834	1.01989	0.45353
16.95	654.47	sea H2O	150	96	0.71025	0.84908	0.21138
16.95	654.47	sea H2O	180	54	1.01936	1.01890	0.62732
16.98	701.60	sea H2O	150	85	3.18993	0.84859	1.08562
16.98	702.46	sea H2O	180	42	1.33561	1.01830	1.07269
17.02	732.83	sea H2O	150	27	0.01235	0.84793	0.01333
17.02	733.63	sea H2O	180	101	0.91505	1.01751	0.30850
17	743.00	sea H2O	250	168	1.25290	1.41376	0.53936
17.09	802.33	sea H2O	200	62	0.87100	1.12904	0.81124
17.13	867.53	sea H2O	250	30	0.45459	1.41020	1.08607
17.06	957.87	sea H2O	200	65	0.54436	1.12969	0.49192
17.04	1073.67	sea H2O	250	82	0.60249	1.41266	0.52194
17.08	1123.60	sea H2O	200	88	0.39548	1.12926	0.26863

VI.3 – *Elminius modestus* larval respiration: different diets, different stages.

"4 algae mixed" is composed by *Pavlova lutheri*, *Rhinomonas reticulata*, *Skeletonema costatum*, and *Tetraselmis chui*

Temperature (°C)	Algal solution	chamber volume (µL)	Larval stage	Larval N	Oxygen drop slope	Max [O2] in chamber (µL Oxigen)	Respiration (nL O2 animal^{-1}hour^{-1})
17.5	4 algae mixed	300	NII	90	6.85602	1.68019	6.74895
17.5	4 algae mixed	300	NII	82	1.03229	1.68019	1.09406
17	4 algae mixed	200	NII	91	7.00906	1.13101	4.50586
17	4 algae mixed	200	NII	47	3.97150	1.13101	5.03929
16.51	4 algae mixed	300	NIII	40	3.74319	1.71277	8.16856
16.51	4 algae mixed	300	NIII	43	2.35856	1.71277	4.73709
16.51	4 algae mixed	200	NIII	130	9.95639	1.14185	4.36111
16.51	4 algae mixed	200	NIII	92	9.46197	1.14185	6.07474
16.52	4 algae mixed	200	NIII	115	6.98429	1.14163	3.43459
16.52	4 algae mixed	200	NIII	69	3.13365	1.14163	2.70840
16.53	4 algae mixed	200	NIII	56	2.61387	1.14140	2.58553
16.53	4 algae mixed	200	NIII	57	5.53516	1.14140	5.95456
16.54	4 algae mixed	200	NIII	63	9.04568	1.14118	7.87260
16.54	4 algae mixed	200	NIII	28	3.15757	1.14118	7.01975
16.52	4 algae mixed	200	NIII	96	8.10377	1.14163	5.35270
16.52	4 algae mixed	200	NIII	77	3.87325	1.14163	2.72429
16.6	4 algae mixed	200	NIV	82	15.09144	1.13984	9.50728
16.6	4 algae mixed	200	NIV	60	6.42446	1.13984	5.88974
16.58	4 algae mixed	200	NIV	67	10.25658	1.14029	7.89006
16.58	4 algae mixed	200	NIV	67	9.23526	1.14029	7.60850
16.61	4 algae mixed	200	NIV	19	3.78700	1.13962	10.23453
16.61	4 algae mixed	200	NIV	36	3.92746	1.13962	6.04062
16.63	4 algae mixed	200	NIV	47	2.05731	1.13918	2.43109
16.63	4 algae mixed	200	NIV	23	2.56896	1.13918	5.71596
16.67	4 algae mixed	200	NIV	81	3.91540	1.13829	2.46562
16.67	4 algae mixed	200	NIV	33	3.94488	1.13829	6.65424

Temperature (°C)	Algal solution	chamber volume (µL)	Larval stage	Larval N	Oxygen drop slope	Max [O2] in chamber (µL Oxigen)	Respiration (nL O2 animal^{-1}hour^{-1})
16.68	4 algae mixed	200	NIV	63	4.24414	1.13807	3.42785
16.68	4 algae mixed	200	NIV	43	3.24500	1.13807	4.21130
15.38	4 algae mixed	150	NV	22	1.25560	0.87566	2.76163
15.37	4 algae mixed	150	NV	27	2.47040	0.87583	4.41703
15.41	4 algae mixed	150	NV	44	2.06872	0.87514	2.25936
14.42	4 algae mixed	150	NV	27	3.16882	0.89262	5.74119
15.42	4 algae mixed	200	NV	14	0.99948	1.16662	4.55469
16.95	4 algae mixed	200	NVI	34	2.81273	1.13211	4.82333
16.95	4 algae mixed	200	NVI	72	3.94439	1.13211	2.78738
17.01	4 algae mixed	200	NVI	36	5.18736	1.13079	7.41609
17.01	4 algae mixed	200	NVI	61	2.29863	1.13079	2.20336
16.29	4 algae mixed	200	NVI	39	1.23873	1.14677	1.85470
16.99	4 algae mixed	200	NVI	45	4.73904	1.13123	5.51273
16.99	4 algae mixed	200	NVI	16	0.50108	1.13123	1.84208
16.26	4 algae mixed	200	NVI	27	1.17315	1.14745	2.55248
16.26	4 algae mixed	150	NVI	14	0.74751	0.86059	2.37835
16.94	4 algae mixed	200	NVI	41	5.82629	1.13233	8.44554
16.94	4 algae mixed	200	NVI	34	3.13121	1.13233	5.62344
16.27	4 algae mixed	200	NVI	61	4.36435	1.14722	4.20972
16.27	4 algae mixed	150	NVI	43	3.03813	0.86042	3.15903
16.25	4 algae mixed	150	NVI	31	5.69613	0.86075	8.24147
16.25	4 algae mixed	200	NVI	41	4.42686	1.14767	6.36383
17.92	*P. lutheri*	150	NII	76	8.30068	0.83335	2.86414
17.9	*P. lutheri*	150	NII	29	3.83508	0.83367	3.48463
17.91	*P. lutheri*	150	NII	146	2.26272	0.83351	0.41213
17.92	*P. lutheri*	150	NII	77	3.66142	0.83335	1.26955
17.92	*P. lutheri*	150	NII	148	4.60302	0.83335	0.83937
17.9	*P. lutheri*	150	NII	95	1.91928	0.83367	0.54769
17.9	*P. lutheri*	150	NIII	61	8.92264	0.83367	4.09327
17.92	*P. lutheri*	150	NIII	17	3.11575	0.83335	5.06635
17.93	*P. lutheri*	150	NIII	23	2.09813	0.83319	2.50696

Temperature (°C)	Algal solution	chamber volume (µL)	Larval stage	Larval N	Oxygen drop slope	Max [O2] in chamber (µL Oxigen)	Respiration (nL O2 animal^{-1}hour^{-1})
17.93	*P. lutheri*	150	NIII	16	0.93739	0.83319	1.60557
17.93	*P. lutheri*	150	NIII	11	2.01764	0.83319	5.00732
17.94	*P. lutheri*	150	NIII	16	0.39161	0.83303	0.66426
17.94	*P. lutheri*	150	NIII	55	2.41040	0.83303	1.18623
17.95	*P. lutheri*	150	NIII	4	0.88391	0.83287	5.95713
17.93	*P. lutheri*	150	NIII	16	2.90632	0.83319	4.87220
17.94	*P. lutheri*	150	NIII	96	1.85583	0.83303	0.51667
17.69	*P. lutheri*	150	NIII	39	4.12933	0.83703	2.83320
16.05	*P. lutheri*	200	NIV	88	1.42385	1.15219	0.94416
15.9	*P. lutheri*	200	NIV	25	3.21583	1.15560	8.33220
15.83	*P. lutheri*	200	NIV	27	3.48610	1.15719	8.18286
16.09	*P. lutheri*	200	NIV	53	1.39951	1.15128	1.56602
16.13	*P. lutheri*	200	NIV	51	2.41276	1.15038	2.84748
16.15	*P. lutheri*	200	NIV	49	1.67556	1.14993	2.10887
15.36	*P. lutheri*	200	NV	11	0.77555	1.16801	4.51817
15.43	*P. lutheri*	200	NV	30	4.66894	1.16638	9.69382
15.2	*P. lutheri*	200	NV	11	1.32566	1.17173	7.46876
15.46	*P. lutheri*	250	NV	7	0.50681	1.45711	5.44394
17.92	*R. reticulata + S. costatum*	180	NII	63	2.03728	1.00002	0.94999
16.5	*R. reticulata + S. costatum*	300	NII	66	3.65709	1.71311	4.90987
16.5	*R. reticulata + S. costatum*	300	NII	88	4.91518	1.71311	5.04532
17.1	*R. reticulata + S. costatum*	200	NII	81	2.70254	1.12882	1.98590
17.1	*R. reticulata + S. costatum*	200	NII	98	3.37346	1.12882	2.00986
17.9	*R. reticulata + S. costatum*	180	NII	15	0.42252	1.00040	0.85770
17.91	*R. reticulata + S. costatum*	180	NII	89	0.84502	1.00021	0.29509
17.87	*R. reticulata + S. costatum*	180	NII	72	2.29826	1.00098	1.01677
16.5	*R. reticulata + S. costatum*	200	NIII	101	7.70745	1.14207	4.37911
16.5	*R. reticulata + S. costatum*	200	NIII	47	6.46669	1.14207	8.04709
16.5	*R. reticulata + S. costatum*	200	NIII	64	2.65271	1.14207	2.35209
16.5	*R. reticulata + S. costatum*	200	NIII	34	4.06147	1.14207	7.09537
16.53	*R. reticulata + S. costatum*	200	NIII	56	3.35264	1.14140	3.33013

Temperature (°C)	Algal solution	chamber volume (µL)	Larval stage	Larval N	Oxygen drop slope	Max [O2] in chamber (µL Oxigen)	Respiration (nL O2 animal^{-1}hour^{-1})
16.53	*R. reticulata* + *S. costatum*	200	NIII	47	3.76709	1.14140	4.88831
16.53	*R. reticulata* + *S. costatum*	200	NIII	13	0.33149	1.14140	1.40609
16.53	*R. reticulata* + *S. costatum*	200	NIII	47	7.12840	1.14140	9.36266
16.53	*R. reticulata* + *S. costatum*	200	NIII	80	6.67314	1.14140	4.54508
16.53	*R. reticulata* + *S. costatum*	200	NIII	28	3.78836	1.14140	8.50209
16.51	*R. reticulata* + *S. costatum*	200	NIII	29	4.50056	1.14185	8.36149
16.51	*R. reticulata* + *S. costatum*	200	NIII	52	3.22215	1.14185	3.96604
16.59	*R. reticulata* + *S. costatum*	200	NIV	113	9.50056	1.14007	4.63037
16.59	*R. reticulata* + *S. costatum*	200	NIV	39	2.85294	1.14007	3.77621
16.6	*R. reticulata* + *S. costatum*	200	NIV	97	14.02218	1.13984	7.99179
16.6	*R. reticulata* + *S. costatum*	200	NIV	46	2.50993	1.13984	2.80617
16.6	*R. reticulata* + *S. costatum*	200	NIV	92	3.31857	1.13984	2.00205
16.6	*R. reticulata* + *S. costatum*	200	NIV	69	3.10899	1.13984	2.30966
16.65	*R. reticulata* + *S. costatum*	200	NIV	42	4.52552	1.13873	5.50414
16.65	*R. reticulata* + *S. costatum*	200	NIV	66	2.80497	1.13873	2.36358
16.69	*R. reticulata* + *S. costatum*	200	NIV	37	4.50166	1.13785	6.78026
16.69	*R. reticulata* + *S. costatum*	200	NIV	86	2.99283	1.13785	1.77203
17	*R. reticulata*	200	NII	177	8.14292	1.13101	2.64461
17.01	*R. reticulata*	250	NII	62	1.96977	1.41349	2.29417
17.09	*R. reticulata*	250	NII	62	1.15449	1.41130	1.34010
17.17	*R. reticulata*	200	NII	82	4.51628	1.12729	3.18801
17.13	*R. reticulata*	200	NII	117	4.53116	1.12816	2.25037
15.48	*R. reticulata*	225	NII	67	1.44795	1.31088	1.57552
15.57	*R. reticulata*	200	NII	92	1.46466	1.16315	1.02912
17.1	*R. reticulata*	250	NII	99	1.96257	1.41102	1.41774
17.06	*R. reticulata*	250	NII	43	1.28322	1.41212	2.13191
15.64	*R. reticulata*	200	NII	57	2.40362	1.16154	2.71967
15.63	*R. reticulata*	200	NII	15	0.22825	1.16177	0.98108
17.02	*R. reticulata*	200	NII	90	3.36980	1.13057	2.21970
15.64	*R. reticulata*	200	NII	78	1.69454	1.16154	1.39935
17.04	*R. reticulata*	200	NII	115	2.04620	1.13013	1.05856

Temperature (°C)	Algal solution	chamber volume (µL)	Larval stage	Larval N	Oxygen drop slope	Max [O2] in chamber (µL Oxigen)	Respiration (nL O2 animal^{-1}hour^{-1})
17.07	*R. reticulata*	250	NII	200	1.71634	1.41184	0.60851
15.67	*R. reticulata*	200	NII	55	0.84682	1.16085	0.99048
17.08	*R. reticulata*	250	NII	114	2.00922	1.41157	1.24795
15.68	*R. reticulata*	200	NII	48	2.81320	1.16062	3.76739
15.71	*R. reticulata*	200	NII	24	0.87822	1.15994	2.34942
15.73	*R. reticulata*	200	NII	39	2.18583	1.15948	3.52722
15.74	*R. reticulata*	200	NII	50	0.50187	1.15925	0.63122
16.9	*R. reticulata*	150	NIII	86	2.08168	0.84990	0.58913
17.12	*R. reticulata*	250	NIII	204	5.59473	1.41048	1.71598
16.8	*R. reticulata*	180	NIII	99	4.79000	1.02187	1.41815
17.1	*R. reticulata*	200	NIII	130	13.16480	1.12882	5.24542
17.07	*R. reticulata*	250	NIII	206	7.58100	1.41184	2.32534
16.8	*R. reticulata*	180	NIII	50	2.55000	1.02187	1.51744
16.8	*R. reticulata*	180	NIII	57	2.12000	1.02187	1.11745
17.08	*R. reticulata*	250	NIII	201	5.32340	1.41157	1.68046
16.8	*R. reticulata*	180	NIII	71	3.86000	1.02187	1.64865
17.13	*R. reticulata*	200	NIII	158	8.26078	1.12816	2.72517
17.07	*R. reticulata*	250	NIII	104	2.33613	1.41184	1.44163
17.12	*R. reticulata*	200	NIII	30	1.72482	1.12838	3.01862
16.98	*R. reticulata*	250	NIII	81	4.31207	1.41431	3.45833
16.8	*R. reticulata*	180	NIII	49	5.20000	1.02187	3.44644
16.87	*R. reticulata*	180	NIV	53	6.73641	1.02048	3.99741
16.82	*R. reticulata*	200	NIV	76	2.37052	1.13497	1.60275
17.02	*R. reticulata*	180	NIV	25	3.01583	1.01751	3.79031
17.04	*R. reticulata*	180	NIV	30	1.00797	1.01712	1.05803
16.78	*R. reticulata*	250	NIV	139	2.63993	1.41982	1.32610
16.03	*R. reticulata*	150	NIV	197	13.16676	0.86448	3.21626
15.9	*R. reticulata*	150	NIV	44	5.41836	0.86670	6.00844
16.95	*R. reticulata*	200	NIV	22	1.83625	1.13211	4.31027
17.04	*R. reticulata*	150	NIV	71	2.66668	0.84760	1.04051
16.97	*R. reticulata*	200	NIV	95	3.08067	1.13167	1.76398

Temperature (°C)	Algal solution	chamber volume (µL)	Larval stage	Larval N	Oxygen drop slope	Max [O2] in chamber (µL Oxigen)	Respiration (nL O2 animal^{-1}hour^{-1})
17.02	*R. reticulata*	180	NIV	26	1.02589	1.01751	1.25228
17.01	*R. reticulata*	200	NIV	14	0.53347	1.13079	1.97950
15.84	*R. reticulata*	150	NIV	17	1.78565	0.86772	5.04425
16.07	*R. reticulata*	150	NIV	38	4.10327	0.86380	5.21262
17.01	*R. reticulata*	200	NIV	114	2.10290	1.13079	0.98739
17.02	*R. reticulata*	180	NIV	66	4.92943	1.01751	2.38032
17.02	*R. reticulata*	180	NIV	60	3.63024	1.01751	1.93260
17.03	*R. reticulata*	180	NIV	38	3.78423	1.01732	3.18550
16.12	*R. reticulata*	150	NIV	59	2.11149	0.86295	1.73163
16.11	*R. reticulata*	150	NIV	50	2.33901	0.86312	2.26607
17.03	*R. reticulata*	180	NIV	35	1.94397	1.01732	1.78095
16.34	*R. reticulata*	150	NV	88	12.49611	0.85924	5.79706
16.34	*R. reticulata*	150	NV	49	3.94076	0.85924	3.31754
16.36	*R. reticulata*	150	NV	30	1.76253	0.85890	2.43622
16.36	*R. reticulata*	150	NV	40	3.73204	0.85890	3.89689
16.36	*R. reticulata*	150	NV	56	5.34670	0.85890	4.00252
16.36	*R. reticulata*	150	NV	38	4.90975	0.85890	5.44260
16.76	*R. reticulata*	200	NVI	77	5.11515	1.13630	3.71657
16.76	*R. reticulata*	200	NVI	60	3.63300	1.13630	3.43591
16.77	*R. reticulata*	200	NVI	82	3.27550	1.13607	2.27141
16.28	*R. reticulata*	200	NVI	9	0.32941	1.14700	2.14029
16.78	*R. reticulata*	200	NVI	82	3.05380	1.13585	2.08715
16.29	*R. reticulata*	200	NVI	28	0.76695	1.14677	1.60305
16.28	*R. reticulata*	200	NVI	38	1.86738	1.14700	2.87913
16.79	*R. reticulata*	200	NVI	81	4.15490	1.13563	2.93653
16.78	*R. reticulata*	200	NVI	74	3.66325	1.13585	2.77925
17.92	*S. costatum*	180	NII	25	0.84478	1.00002	1.01374
17.9	*S. costatum*	180	NII	29	0.77492	1.00040	0.80695
17.91	*S. costatum*	180	NII	82	0.37603	1.00021	0.14030
17.92	*S. costatum*	180	NII	83	1.04992	1.00002	0.38934
17.92	*S. costatum*	180	NII	61	1.54004	1.00002	0.78872

Temperature (°C)	Algal solution	chamber volume (µL)	Larval stage	Larval N	Oxygen drop slope	Max [O2] in chamber (µL Oxigen)	Respiration (nL O2 animal^{-1}hour^{-1})
17.9	*S. costatum*	180	NII	24	0.38885	1.00040	0.50926
17.9	*S. costatum*	180	NIII	43	2.69020	1.00040	1.97146
17.92	*S. costatum*	180	NIII	56	4.64226	1.00002	2.61848
17.93	*S. costatum*	180	NIII	17	0.31715	0.99983	0.59005
17.93	*S. costatum*	180	NIII	7	0.41389	0.99983	1.87142
17.93	*S. costatum*	180	NIII	75	0.86159	0.99983	0.36397
17.94	*S. costatum*	180	NIII	40	0.55994	0.99964	0.44413
17.94	*S. costatum*	180	NIII	63	1.12849	0.99964	0.56871
17.95	*S. costatum*	180	NIII	55	4.46268	0.99944	2.57833
17.93	*S. costatum*	180	NIII	42	6.82170	0.99983	5.17064
17.94	*S. costatum*	180	NIII	44	0.87599	0.99964	0.63428
17.69	*S. costatum*	180	NIII	75	0.90948	1.00444	0.38858
16.05	*S. costatum*	150	NIV	111	10.23088	0.86414	4.44030
15.9	*S. costatum*	150	NIV	45	7.54878	0.86670	8.12597
15.83	*S. costatum*	150	NIV	43	8.51691	0.86789	9.45490
16.09	*S. costatum*	150	NIV	52	3.13957	0.86346	2.91887
16.13	*S. costatum*	150	NIV	57	1.30929	0.86278	1.11398
16.15	*S. costatum*	150	NIV	96	5.13728	0.86245	2.60983
15.36	*S. costatum*	150	NV	60	3.35578	0.87600	2.70414
15.43	*S. costatum*	150	NV	100	17.01722	0.87479	8.16562
15.2	*S. costatum*	150	NV	70	11.62190	0.87879	7.98574
15.46	*S. costatum*	150	NV	90	13.55248	0.87427	7.16350
17.92	*T. chui*	150	NII	100	2.82158	0.83335	0.72854
17.9	*T. chui*	150	NII	52	3.86022	0.83367	1.96797
17.91	*T. chui*	150	NII	180	1.08878	0.83351	0.16271
17.87	*T. chui*	150	NII	82	5.01846	0.83415	1.67537
17.91	*T. chui*	150	NIII	16	0.74094	0.83351	1.27642
17.91	*T. chui*	180	NIII	45	0.70687	1.00021	0.49667
17.94	*T. chui*	150	NIII	66	1.86587	0.83303	0.76964
17.94	*T. chui*	180	NIII	92	1.21150	0.99964	0.41743
17.94	*T. chui*	150	NIII	10	0.43361	0.83303	1.16673

Temperature (°C)	Algal solution	chamber volume (µL)	Larval stage	Larval N	Oxygen drop slope	Max [O2] in chamber (µL Oxigen)	Respiration (nL O2 animal^{-1}hour^{-1})
17.94	*T. chui*	180	NIII	27	1.10903	0.99964	1.30622
17.86	*T. chui*	150	NIII	59	7.31297	0.83431	3.29496
17.86	*T. chui*	180	NIII	95	2.93344	1.00117	0.98715
16.03	*T. chui*	200	NIV	90	2.74929	1.15264	1.77271
15.9	*T. chui*	220	NIV	16	1.79350	1.27116	8.07040
15.84	*T. chui*	200	NIV	16	1.88590	1.15696	7.53785
16.07	*T. chui*	200	NIV	46	1.67937	1.15174	2.14977
16.12	*T. chui*	200	NIV	37	3.25506	1.15060	5.24479
16.11	*T. chui*	200	NIV	27	1.99401	1.15083	4.42517
16.15	*T. chui*	200	NIV	48	5.02908	1.14993	6.41714
15.38	*T. chui*	200	NV	40	5.27939	1.16754	8.50312
15.37	*T. chui*	200	NV	10	0.47854	1.16777	3.04856
15.41	*T. chui*	200	NV	67	3.52979	1.16685	3.29890
14.42	*T. chui*	200	NV	24	1.63659	1.19016	4.31718
15.42	*T. chui*	250	NV	53	2.88969	1.45827	4.19202

VI.4 – Oxygen consumption of *Elminius modestus* larvae feeding and non-feeding on *Rhinomonas reticulata.*

Temperature (°C)	experimental time (minutes)	food yes/no	chamber volume (µL)	Larval stage	Larval N	Oxygen drop slope	Max [O2] in chamber (µL Oxigen)	Respiration (nL O2 animal^{-1}hour^{-1})
17.01	27.87	no	200	NII	42	0.85236	1.13079	1.16950
17.17	101.07	no	250	NII	101	3.21985	1.40911	2.28630
15.48	132.77	no	225	NII	39	0.80845	1.31088	1.51492
15.57	176.82	no	150	NII	51	0.53690	0.87236	0.51266
17.1	179.13	no	250	NII	57	1.47084	1.41102	1.88538
15.64	234.08	no	150	NII	74	0.37989	0.87116	0.25007
15.63	267.72	no	150	NII	54	0.28689	0.87133	0.25911
17.02	295.73	no	250	NII	114	1.24907	1.41321	0.78016
15.64	317.65	no	150	NII	37	0.52182	0.87116	0.68873
17.07	358.33	no	200	NII	188	2.33580	1.12947	0.74077
15.67	360.25	no	150	NII	45	0.56868	0.87064	0.61756
15.68	400.20	no	150	NII	15	0.22262	0.87047	0.72600
15.71	435.33	no	150	NII	60	0.46126	0.86995	0.37623
16.9	3.13	no	180	NIII	170	7.10000	1.01989	1.20580
17.12	5.00	no	200	NIII	144	9.41680	1.12838	3.37600
16.8	37.54	no	150	NIII	88	1.35748	0.85156	0.37832
17.1	45.00	no	250	NIII	184	1.21420	1.41102	0.41523
17.07	70.67	no	200	NIII	99	5.23426	1.12947	2.74539
16.8	75.95	no	150	NIII	66	1.18040	0.85156	0.44155
16.8	102.39	no	150	NIII	33	1.07500	0.85156	0.80796
17.08	105.50	no	200	NIII	156	6.63640	1.12926	2.21425
16.8	125.77	no	150	NIII	33	0.15984	0.85156	0.12063
17.13	138.20	no	250	NIII	30	0.95740	1.41020	2.03227
17.07	187.73	no	200	NIII	30	1.30062	1.12947	2.27082
17.12	232.93	no	250	NIII	92	0.99213	1.41048	0.69579
16.98	263.33	no	200	NIII	75	1.48014	1.13145	1.04138
16.8	294.76	no	150	NIII	61	0.31058	0.85156	0.13067

Temperature (°C)	experimental time (minutes)	food yes/no	chamber volume (µL)	Larval stage	Larval N	Oxygen drop slope	Max [O2] in chamber (µL Oxigen)	Respiration (nL O2 animal^{-1}hour^{-1})
16.87	1.93	no	150	NIV	54	4.99520	0.85040	2.50885
17.02	28.36	no	150	NIV	37	1.92466	0.84793	1.41464
16.82	29.67	no	200	NIV	78	3.59367	1.13497	2.60237
17.04	61.98	no	150	NIV	29	0.90875	0.84760	0.85802
16.78	69.33	no	200	NIV	33	2.38127	1.13585	3.72633
16.95	101.07	no	200	NIV	38	0.69907	1.13211	1.01460
17.04	114.58	no	180	NIV	25	3.13938	1.01712	3.97033
16.97	145.87	no	200	NIV	44	2.55369	1.13167	3.01017
17.02	157.13	no	150	NIV	33	1.03285	0.84793	0.87524
17.01	168.40	no	200	NIV	117	0.15970	1.13079	0.07372
17.01	198.87	no	200	NIV	177	7.21709	1.13079	2.12503
17.02	210.20	no	150	NIV	31	3.58991	0.84793	3.27653
17.02	235.90	no	150	NIV	10	0.18607	0.84793	0.52949
17.03	258.09	no	150	NIV	68	1.85417	0.84776	0.77964
17.03	289.62	no	150	NIV	48	0.53722	0.84776	0.32229
16.34	10.27	no	200	NV	44	0.78692	1.14565	1.04466
16.34	86.90	no	200	NV	37	0.86431	1.14565	1.36845
16.36	128.15	no	200	NV	90	3.77831	1.14520	2.46223
16.36	180.26	no	200	NV	89	2.05493	1.14520	1.35688
16.36	207.46	no	200	NV	43	0.69141	1.14520	0.94592
16.36	241.95	no	200	NV	88	2.47623	1.14520	1.65755
16.76	0.00	no	200	NVI	45	1.22013	1.13630	1.53381
16.29	33.38	no	150	NVI	8	0.22958	0.86008	1.26212
16.76	34.33	no	200	NVI	19	0.89800	1.13630	2.64628
16.77	60.06	no	200	NVI	52	0.54753	1.13607	0.58977
16.28	73.28	no	150	NVI	14	0.94513	0.86025	2.97807
16.78	94.34	no	200	NVI	63	0.57221	1.13585	0.51803
16.29	103.63	no	150	NVI	44	1.57868	0.86008	1.58588
16.28	131.33	no	150	NVI	21	0.75055	0.86025	1.58319
16.79	138.06	no	200	NVI	60	1.24113	1.13563	1.16025

Temperature (°C)	experimental time (minutes)	food yes/no	chamber volume (µL)	Larval stage	Larval N	Oxygen drop slope	Max [O2] in chamber (µL Oxigen)	Respiration (nL O2 animal^{-1}hour^{-1})
16.78	171.27	no	200	NVI	26	1.07310	1.13585	2.37065
17	2.40	yes	200	NII	177	8.14292	1.13101	2.64461
17.01	28.87	yes	250	NII	62	1.96977	1.41349	2.29417
17.09	63.67	yes	250	NII	62	1.15449	1.41130	1.34010
17.17	99.60	yes	200	NII	82	4.51628	1.12729	3.18801
17.13	128.87	yes	200	NII	117	4.53116	1.12816	2.25037
15.48	132.37	yes	225	NII	67	1.44795	1.31088	1.57552
15.57	176.82	yes	200	NII	92	1.46466	1.16315	1.02912
17.1	180.87	yes	250	NII	99	1.96257	1.41102	1.41774
17.06	216.80	yes	250	NII	43	1.28322	1.41212	2.13191
15.64	234.95	yes	200	NII	57	2.40362	1.16154	2.71967
15.63	267.98	yes	200	NII	15	0.22825	1.16177	0.98108
17.02	296.67	yes	200	NII	90	3.36980	1.13057	2.21970
15.64	318.05	yes	200	NII	78	1.69454	1.16154	1.39935
17.04	333.00	yes	200	NII	115	2.04620	1.13013	1.05856
17.07	357.60	yes	250	NII	200	1.71634	1.41184	0.60851
15.67	362.25	yes	200	NII	55	0.84682	1.16085	0.99048
17.08	381.80	yes	250	NII	114	2.00922	1.41157	1.24795
15.68	399.33	yes	200	NII	48	2.81320	1.16062	3.76739
15.71	437.47	yes	200	NII	24	0.87822	1.15994	2.34942
16.9	4.47	yes	150	NIII	86	2.08168	0.84990	0.58913
17.12	5.53	yes	250	NIII	204	5.59473	1.41048	1.71598
16.8	38.07	yes	180	NIII	99	4.79000	1.02187	1.41815
17.1	45.00	yes	200	NIII	130	13.16480	1.12882	5.24542
17.07	71.80	yes	250	NIII	206	7.58100	1.41184	2.32534
16.8	77.35	yes	180	NIII	50	2.55000	1.02187	1.51744
16.8	102.52	yes	180	NIII	57	2.12000	1.02187	1.11745
17.08	104.20	yes	250	NIII	201	5.32340	1.41157	1.68046
16.8	126.30	yes	180	NIII	71	3.86000	1.02187	1.64865
17.13	137.53	yes	200	NIII	158	8.26078	1.12816	2.72517

Temperature (°C)	experimental time (minutes)	food yes/no	chamber volume (µL)	Larval stage	Larval N	Oxygen drop slope	Max [O2] in chamber (µL Oxigen)	Respiration (nL O2 animal^{-1}hour^{-1})
17.07	186.93	yes	250	NIII	104	2.33613	1.41184	1.44163
17.12	232.47	yes	200	NIII	30	1.72482	1.12838	3.01862
16.98	262.87	yes	250	NIII	81	4.31207	1.41431	3.45833
16.8	295.16	yes	180	NIII	49	5.20000	1.02187	3.44644
16.87	4.40	yes	180	NIV	53	6.73641	1.02048	3.99741
16.82	29.40	yes	200	NIV	76	2.37052	1.13497	1.60275
17.02	29.50	yes	180	NIV	25	3.01583	1.01751	3.79031
17.04	63.05	yes	180	NIV	30	1.00797	1.01712	1.05803
16.78	69.33	yes	250	NIV	139	2.63993	1.41982	1.32610
16.03	80.64	yes	150	NIV	197	13.16676	0.86448	3.21626
15.9	83.46	yes	150	NIV	44	5.41836	0.86670	6.00844
16.95	101.07	yes	200	NIV	22	1.83625	1.13211	4.31027
17.04	116.05	yes	150	NIV	71	2.66668	0.84760	1.04051
16.97	146.67	yes	200	NIV	95	3.08067	1.13167	1.76398
17.02	158.06	yes	180	NIV	26	1.02589	1.01751	1.25228
17.01	168.33	yes	200	NIV	14	0.53347	1.13079	1.97950
15.84	177.49	yes	150	NIV	17	1.78565	0.86772	5.04425
16.07	181.45	yes	150	NIV	38	4.10327	0.86380	5.21262
17.01	199.60	yes	200	NIV	114	2.10290	1.13079	0.98739
17.02	211.00	yes	180	NIV	66	4.92943	1.01751	2.38032
17.02	239.43	yes	180	NIV	60	3.63024	1.01751	1.93260
17.03	260.15	yes	180	NIV	38	3.78423	1.01732	3.18550
16.12	265.58	yes	150	NIV	59	2.11149	0.86295	1.73163
16.11	289.42	yes	150	NIV	50	2.33901	0.86312	2.26607
17.03	290.55	yes	180	NIV	35	1.94397	1.01732	1.78095
16.15	438.82	yes	150	NIV	42	1.04657	0.86245	1.21326
16.34	9.73	yes	150	NV	88	12.49611	0.85924	5.79706
16.34	86.50	yes	150	NV	49	3.94076	0.85924	3.31754
16.36	127.62	yes	150	NV	30	1.76253	0.85890	2.43622
16.36	180.06	yes	150	NV	40	3.73204	0.85890	3.89689

Temperature (°C)	experimental time (minutes)	food yes/no	chamber volume (µL)	Larval stage	Larval N	Oxygen drop slope	Max [O2] in chamber (µL Oxigen)	Respiration (nL O2 animal^{-1}hour^{-1})
16.36	206.80	yes	150	NV	56	5.34670	0.85890	4.00252
16.36	241.55	yes	150	NV	38	4.90975	0.85890	5.44260
16.76	0.00	yes	200	NVI	77	5.11515	1.13630	3.71657
16.76	34.27	yes	200	NVI	60	3.63300	1.13630	3.43591
16.77	59.27	yes	200	NVI	82	3.27550	1.13607	2.27141
16.28	72.55	yes	200	NVI	9	0.32941	1.14700	2.14029
16.78	93.33	yes	200	NVI	82	3.05380	1.13585	2.08715
16.29	104.43	yes	200	NVI	28	0.76695	1.14677	1.60305
16.28	131.87	yes	200	NVI	38	1.86738	1.14700	2.87913
16.79	137.27	yes	200	NVI	81	4.15490	1.13563	2.93653
16.78	170.66	yes	200	NVI	74	3.66325	1.13585	2.77925