

Author version: J. Exp. Mar. Biol. Ecol., vol.392(1-2); 2010; 89-98

## **Larval development, sensory mechanisms and physiological adaptations in acorn barnacles with special reference to *Balanus amphitrite***

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### **ABSTRACT**

Barnacles have drawn the attention of many naturalists and often dominate fouling communities. *Balanus amphitrite*, is a shallow water acorn barnacle capable of inhabiting expanses from supralittoral to subtidal levels, and as an epibiont. Its potential to survive and successfully establish local population is endorsed by various physiological adaptations and larval sensory perceptions. The larval life cycle of this species has both planktotrophic naupliar and non-feeding cypris stages. The naupliar energetics has a bearing on the capabilities of cypris larvae to explore surfaces for settlement and also the recruitment success of juveniles. The most complete nervous system in the barnacles is established in the cypris larva. Although there has been considerable research with reference to their settlement and metamorphosis, not much is known about the olfactory, photo and auditory sensory mechanisms with respect to settlement and metamorphosis, which need further attention. Understanding the response of most sensitive life stages of barnacles to environmental changes in intertidal habitats can also serve as important models for understanding the effect of climate change on species distribution.

**Keywords:** Barnacle; Biofouling; *Balanus amphitrite*; nauplii; cypris; energetics; physiology; settlement cues

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## Introduction

Barnacles are one of the most dominant fouling organisms and an important component of the intertidal community. Among the barnacles, *Balanus amphitrite* is utilized as a candidate organism in antifouling research and hard substratum benthic ecology study, owing to well established rearing techniques. Darwin (1854) classified *B. amphitrite* complex into nine varieties namely *communis*, *venustus*, *pallidus*, *niveus*, *modestus*, *stutsburi*, *obscurus*, *variegatus* and *cirratus*. Since none of the original varieties had type specimen, a revised nomenclature was proposed by Harding (1962) by assigning lectotypes for the varieties of Darwin's dried specimens. He divided Darwin's nine varieties into four separate species: *B. amphitrite*, *B. pallidus*, *B. venustus* and *B. variegatus*. *Balanus amphitrite communis* was placed into *B. amphitrite* var. *amphitrite*. The varieties *pallidus* and *stutsburi* were placed into species *pallidus*. Darwin's varieties *venustus*, *niveus*, *modestus* and *obscurus* were placed in the species *venustus*; the varieties *variegatus* and *cirratus* were placed under the species *variegatus*. He also synonymized Broch's (1927) variety *denticulata* with *B. amphitrite amphitrite*. It was revealed by Utimoni (1967) and further verified by Southward (1975) that Darwin had placed two species of *B. amphitrite* under the same taxa; *B. amphitrite* and *B. reticulatus*. Advancement in the understanding of the taxonomy of *B. amphitrite* group came through an extensive study of their morphology by Henry and McLaughlin (1975) and it was concluded that only two subspecies exist, *B. amphitrite amphitrite* and *B. amphitrite saltonensis*. Based on genetic analysis, Flowerdew (1985), argued for placing *amphitrite* variety *saltonensis* in synonymy with the variety *amphitrite*. Pitombo (2004) carried out a major phylogenetic revision of Balanidae that resulted in a new family Amphibalanidae. As a consequence, *B. amphitrite* was renamed as *Amphibalanus amphitrite*. Recently, Clare and Hoeg (2008) suggested that the introduction of *Amphibalanus* would seriously confuse the naming of a barnacle that is at the centre of experimental research and suggested retention of the earlier nomenclature or adoption of a compromise nomenclature. However, in a reply to this suggestion Carlton and Newman (2009) stated that the new name, *Amphibalanus amphitrite* was proposed in accordance with the International Code of Zoological Nomenclature. They also pointed out that the criticism offered by Clare and Hoeg (2008) had no scientifically valid reason to return to the earlier nomenclature of this or any other well-known species of barnacle. In view of this, though we have used *Balanus amphitrite* in this paper, we wish to state that it is without any prejudice to the debated taxonomic status. This review provides an overview of the research carried out in relation to larval development, cyprid energetics, sensory mechanisms and physiological adaptations in acorn barnacles with special reference to *B. amphitrite*.

## Larval development

Most of the balanomorph barnacles are simultaneous hermaphrodites (Charnov, 1987), for which obligate internal cross-fertilization is the norm. However, incidences of self-fertilization have also been reported (Barnes and Crisp, 1956; Furman and Yule, 1990; El-Komi and Kajihara, 1991; Desai et al., 2006). Experiments with *B. amphitrite* indicated that egg production was high with brood intervals of 5-8 days per brood compared to boreo-arctic species which usually produce a single brood per year (Crisp and Davies, 1955; El-Komi and Kajihara, 1991). Temperature and nutritional conditions also influence breeding and molting processes in this species. Field observations in a tropical coastal environment influenced by monsoon also point out a positive relationship between gonad development of *B. amphitrite* and chlorophyll *a* concentration (Desai et al., 2006). Documenting reproductive hotspots along the Oregon Coast (USA), Leslie et al. (2005) pointed out that the intertidal barnacle *Balanus glandula* population in the region of higher primary productivity produced almost five times more offspring than those in the regions of lower productivity. Barnes and Barnes (1958) concluded that availability of the 'right' food type is of fundamental importance in the development of planktonic larvae with high metabolic rates and suggested that food may interact with and compensate for the effect of temperature. It was pointed out that, when starved adults of *Semibalanus balanoides* retain egg masses beyond the 'normal' hatching time, egg metabolism occurs largely at the expense of remaining lipid reserves (Lucas and Crisp, 1987). In general, eggs of marine species use lipids as their main energy source followed by proteins and carbohydrates (Pandian, 1969, 1970). Thus, the ability of adults to postpone hatching may have important implications for the energy reserves and viability of newly hatched nauplii. Whenever retention period is reasonably short, each newly hatched larva appears to possess enough energy to start the pelagic life (Lucas and Crisp, 1987). Experiments with the nauplii of *B. amphitrite*, that were hatched from adults collected during different seasons indicated that larvae obtained during late autumn to early spring had poor capability to develop when compared to larvae collected during the summer months. It was pointed out that generally, during late autumn to early spring, less food is available for adults; hence, retention of egg mass within the adult would be at the cost of its nutritional status (Anil et al., 1995). Thus, in balanomorph barnacles energy metabolism of eggs has a vital role in their larval ecology.

Olson and Olson (1989) assessed food limitation of planktotrophic invertebrate larvae and concluded that larval starvation is likely to be important to recruitment success in cirripedes. Studies on

the specific mechanisms effecting starvation sensitivity in the larval development of decapod crustaceans suggested a positive interaction between food availability and endogenous hormonal control of development (Anger, 1987). Furthermore, when an early larva is starved beyond a certain point, its feeding ability decreases and it eventually experiences irreversible damage, probably to the mitochondria and hepatopancreas system (Storch and Anger, 1983). Scheltema and Williams (1982) proposed that reduced feeding efficiency of *B. eburneus* nauplii reared at low experimental temperature can be compensated by increased algal cell concentration. *B. amphitrite* larval development is euryhaline and observations revealed that the temperature influence is greatest on the instar II nauplii (Anil et al., 1995). It was further observed that food availability and temperature jointly determine energy allocation for development and the influence varied with naupliar instars. The influence of salinity (15, 25 and 30 ) at a given food concentration ( $0.5, 1$  and  $2 \times 10^5$  cells  $\text{ml}^{-1}$  of *Skeletonema costatum*) on instar IV, V, VI and total naupliar duration was negligible at  $20^\circ\text{C}$ , while at  $30^\circ\text{C}$  there was marked decrease in duration with increasing salinity (Anil and Kurian, 1996).

It has also been observed that *B. amphitrite* naupliar swimming rate significantly increases with increasing temperature (Yule, 1984). He hypothesized that, if the larvae swim faster due to increase in temperature, a greater percentage of their available energy may go into swimming, and the ability of the larvae to replace that energy becomes a limiting factor for continued larval development. Podolsky (1994) manipulated sea water viscosity at various temperatures, to distinguish the physiological and mechanical effects of temperature on suspension feeding by ciliated echinoderm larvae and found that the increased viscosity alone accounted for half of the decline in feeding rate at lower temperatures. High viscosity shifted ingestion towards larger particles, which suggests that viscosity affects particle capture and rates of water processing (Podolsky, 1994). Laboratory studies on the nauplii of *Balanus improvisus*, revealed that total starvation suppressed molting beyond stage II; 50% mortality occurred in approximately 4 days at both  $15$  and  $21^\circ\text{C}$ , while the longest survival time was 7 days at  $15^\circ\text{C}$  and 6 days at  $21^\circ\text{C}$  (Lang and Marcy, 1982). Susceptibility of *B. amphitrite* nauplii to starvation varied significantly with the rearing temperature. Nauplii starved at  $5^\circ\text{C}$  had Ultimate Recovery Point (URP) of 204 h (URP, denotes the starvation point in hours at the end of which a larva can recover and continue development). The URP reduced to 60 and 24 h when the nauplii were starved at  $15$  and  $24^\circ\text{C}$  respectively (Desai and Anil, 2000).

In nature, larvae fulfill their energy demands from a soup of available food material, while those reared in the laboratory are typically provided with a suitable diet. In order to understand the variations between laboratory and field reared larvae, instar II nauplii of *Balanus amphitrite* were reared in the field using micro enclosures (1 liter capacity PVC bottles, which were cut at the sides as well as the bottom, sealing the cut portion with 100 $\mu$ m mesh using glue, and ensuring that they are leak proof). The micro enclosures were suspended in the water column from the jetty at a depth of 1 m below the lowest low tide mark. After every 24 h, nauplii were washed into glass beakers and about 25 nauplii were siphoned out and the larval size and stage was recorded and were measured for RNA and DNA content. Results from this study showed that larvae reared in the laboratory had approximately 1.7 times higher RNA: DNA ratio (as a nutritional status indicator) than those raised at a comparable temperature in the field. Naupliar duration for laboratory reared larva was two days shorter (Desai and Anil, 2002). Chlorophyll *a* values during field rearing ranged from 1.9 to 4 $\mu$ g l<sup>-1</sup>, whereas chlorophyll *a* content of food provided in the laboratory (1x10<sup>5</sup> cells l<sup>-1</sup> of *S. costatum*) was 60 $\mu$ g l<sup>-1</sup>. Even at the lowest laboratory rearing concentrations, the chlorophyll *a* content was 15 times higher than the maximum value found in the field. It is also to be noted that the natural phytoplankton population includes forms, which are of no value as cirripede larval feed. Owing to such differences, the status of the larvae in the field and those raised in the laboratory can be widely different.

Another important factor that influences larval development is the naupliar feeding mechanism. Interpretation of cirripede naupliar feeding mechanism is largely based on anatomy and limb motions during swimming and grooming rather than direct observations of particle capture. Studies show that the gnathobases of the second antennae are used to ingest particles, but a variety of mechanisms are involved in the concentration of particles and in transporting them to mouth (Strathmann, 1987; Vargas et al., 2006). Food clearance rate in feeding is proportional to the length of ciliated band (Strathmann, 1971; Strathmann et al., 1972), which commonly increases with increasing larval body length (Strathmann and Bonar, 1976; McEdward, 1984). It was pointed out that intersetular distance of cirripede nauplii is likely to increase with successive molts (Stone, 1986, 1988). This increase in mesh size can influence the size of food particles that larvae capture (Stone, 1988). Thus grazing rate would vary ontogenetically and with food type. Exemplifying this, it has been found that early naupliar instars of *B. amphitrite* had higher grazing rates when fed with single cell diatom *Cheatoceros calcitrans* (4-6  $\mu$ m) whereas in the case of advanced instars it was chain forming *S. costatum* (4-12  $\mu$ m in diameter).

Early instar larvae recovered better from starvation when fed with *C. calcitrans* whereas advanced instars recovered better when fed with *S. costatum* (Desai and Anil, 2004).

Release of barnacle larvae in synchrony with major algal blooms has been identified as a trade-off between adequate food supply for larvae and renewal of adult reserves (Starr et al., 1991). Earlier, it was also observed that the release of *Semibalanus balanoides* nauplii was closely associated with the spring *S. costatum* outburst at Millport, Scotland (Barnes, 1962). In regions or situations where seasonal changes are clearly distinct such a signal can work in favor of successful recruitment. It has also been suggested that persistent changes in the structure of pelagic food webs can have important effects on the species-specific food availability for invertebrate larvae, which can result in large-scale differences in recruitment rates of a given species, and in the relative recruitment success of the different species that make up benthic communities (Vargas et al., 2006). Studies carried out in a tropical estuarine environment influenced by South-West monsoon along the West Coast of Indian subcontinent on the settlement of barnacles brought out the vagaries involved in cue synchronized larval release. In this particular study (Desai and Anil, 2005), it was observed that larvae released in response to phytoplankton blooms (Patil and Anil, 2008) during intraseasonal breaks in monsoon resulted in settlement peaks of *B. amphitrite*. However, the individuals that settled during monsoon break period were stressed with the recurrence of monsoon conditions resulting in recruitment failure. Taking this into consideration it is possible to say that inappropriate cue synchronization can cause perturbations to recruitment in environments influenced by monsoon.

Sanford and Menge (2001) evaluated spatial and temporal variation in barnacle growth in a coastal upwelling system on the central Oregon coast (USA) and pointed out that factors other than phytoplankton contributed to variation in barnacle growth. Their observations revealed that increased barnacle growth during the upwelling relaxation would have resulted from the combined benefits of increased phytoplankton, zooplankton, and warmer water temperatures. In turn they suggested that it is important to incorporate the influence of zooplankton and water temperature into studies of bottom-up influences to explain variations among the intertidal communities. Preliminary results on settlement of the barnacles *Tetraclita stalactifera* and *Chthamalus bisinuatus* on a Brazilian tropical rocky shore under upwelling conditions pointed out that in the summer, densities of cyprids and settlement rates of both species were low, whereas densities of nauplii were high. Whereas, in autumn/winter, high densities of cyprids and high settlement rates were found, while densities of nauplii were lower than

cyprids. It was pointed that this could be due to higher water temperature that can accelerate larval metamorphosis, and/or due to winter storms that may increase transport to shore. These results along the coast of Brazil contrast with those from other upwelling regions where settlement is highest during spring/summer (Skinner and Coutinho, 2002). Understanding synchrony of larval release with algal blooms attains greater importance in view of climate change scenario and occurrence of the rising trends in frequency and magnitude of extreme rain events. Implications of such events on invertebrate reproductive biology, plankton dispersion, settlement and recruitment need a fresh look. Variation in the influence of such factors on different taxa can have far reaching effects on community structure in a given environment.

In the wild, the food filtered by barnacle larvae includes small flagellates at relatively high rates along with diatoms (Turner et al., 2001). It has also been observed that barnacle nauplii can also feed on autotrophic picoplankton (<5  $\mu\text{m}$ ) and not consume the larger phytoplankton cells (Vargas et al., 2006). Experiments have also indicated that barnacle nauplii can feed on *Phaeocystis* (Nejstgaard et al., 2007). In a study carried out in a tropical monsoon influenced bay indicated that the consistency in settlement and recruitment observed during the pre-monsoon season coincided with the higher percentage of defaecating larvae and the absence of diatom frustules in the fecal pellets (Gaonkar and Anil, 2009). These results suggest the importance of non diatoms in the food of cirripede nauplii. In this regard further studies on the quantitative analysis of the production and content of fecal pellets could provide valuable insights to the larval food types in the wild. Application of relevant molecular techniques (Nejstgaard et al., 2003; Vestheim et al., 2005; Blankenship and Yayanos, 2005) or estimation of stable isotopic signatures (Buskey et al., 1999, Tamelander et al., 2006, El-Sabaawi et al., 2009) of the larvae or their fecal pellets to identify different types of food ingested can be a step forward in the larval nutritional ecology. Larval development success in the natural environment in spite of altered food availability also has implications on their dispersion to different habitats and the energetics of non feeding cypris larva.

### **Cyprid energetics**

Variations in the settlement behavior of cypris larva is influenced by the energy reserves (Lucas et al., 1979; Anil and Kurian, 1996; Pechenik et al., 1998; Anil et al., 2001; Thiyagarajan et al., 2002; Desai and Anil, 2004 and references therein). Larval size also influences larval life span in some

invertebrates, with larger larva remaining active for longer than the smaller ones (Marshall et al., 2003; Marshall and Keough, 2003; Isomura and Nishihira, 2001). If larval size affects larval settlement behavior, then variation in larval size could also indirectly affect dispersal potential of the larva. Larger larva will have greater nutritional reserves than the smaller larva; larger larva can swim for longer duration and would be less desperate to settle than the smaller larva. In this context, it is possible for larger larva to remain planktonic for longer duration, hence a greater dispersal potential.

In planktotrophic forms, planktonic period and attainment of a competence stage is variable. In the absence of cues, the capacity to maintain that larval state decreases with time, often leading to decreased discrimination or spontaneous metamorphosis (desperate larva hypothesis). Alternatively, larva may fail to metamorphose and subsequently die as larva, a process termed “variable retention hypothesis” or “death before dishonor hypothesis” (Bishop et al., 2006a). The only concrete positive metamorphosis cue illustrated in the case of *B. amphitrite* is arthropodin (glycoprotein) which is produced by conspecific adults (Knight-Jones, 1953; Knight-Jones and Crisp, 1953; Crisp and Meadows, 1963), although data suggest that cue specificity in *B. amphitrite* is rather poor (Bishop et al., 2006a). Most planktotrophic larvae become competent to metamorphose only after spending some time in the plankton (Bishop et al., 2006b). The definition of metamorphosis has been debated with habitat shift and major morphological changes as central criteria in the definition (Bishop et al., 2006a). Thus cyprid transformation to a juvenile (‘spat’) is usually considered as metamorphosis. The term “delay of metamorphosis” is used extensively to reflect plasticity in the developmental process (Pechenik, 1990). Metamorphosis is a dynamic, environmentally dependent process that integrates ontogeny with habitat. The capability to maintain metamorphic competence in the absence of environmental cues has also been hypothesized to be an adaptive strategy (Hadfield et al., 2001). It has also been pointed out that as cyprids grow old, they become less discriminatory in their selection of settlement substrate (Knight-Jones, 1953; Toonen and Pawlik, 2001).

Even after successful metamorphosis, competitive ability of the juveniles can be impaired by reduced growth rate which is dependent upon larval energetics (Jarrett and Pechenik, 1997; Jarrett, 2003). Pechenik et al. (1993) observed that delayed metamorphosis in *B. amphitrite* increases an individual’s chance of locating a site appropriate for metamorphosis but simultaneously reduces the ability to compete for space within the first few weeks of juvenile life.

Cyprid Major Protein (CMP) has been described as a storage protein that is biochemically and immunologically similar to vitellin. This protein, accumulates during naupliar stages, is most abundant in the cyprid, and abruptly decreases in quantity following metamorphosis to the juvenile barnacle (Shimizu et al., 1996). It was also reported that newly molted cyprids require some time to become competent and CMP utilization during the initial phase (up to three days) may lead to competency. It was further pointed out that depletion of CMP is responsible for reduction in settlement success such that the remaining CMP stores can no longer support the production of adult structure following settlement (Satuito et al., 1996). In another study (Anil et al., 2001) the influence of naupliar experiences on cyprid metamorphosis was quantified through estimation of RNA/DNA ratio (as an indicator of nutritional condition) and found that the RNA content of *B. amphitrite* nauplii raised at 20°C was considerably less than in nauplii raised at 30°C. This difference in RNA content influenced the cyprid settlement capability. An increase in naupliar rearing temperature from 20°C to 30°C, increased the potential of cyprids to survive and settle. Cyprids obtained at 20°C could successfully metamorphose for 2-4 days; whereas those obtained at 30 °C could successfully metamorphose over 8-16 days (Anil et al., 2001). In this context, it is pertinent to note that cypris larvae are generally stored at 5°C for 2 days (Rittschof et al., 1992) prior to carrying out settlement assays in order to condition them for settlement. However the effect of such conditioning on larvae raised at different temperatures is yet to be elucidated.

Cyprid energy reserve measured as ratio of Triacylglycerols (TAG) to DNA is also useful in identifying the discriminatory metamorphic behavior of cyprids (Thiyagarajan et al., 2002). They observed that cyprids with high energy reserves were indifferent to settlement factor or adult extract, indicating that cyprids may attach solitarily to form new colonies or they may settle gregariously in close proximity to conspecifics. Further they also observed that, cyprids with low energy reserves might distinctively attach gregariously due to a more pronounced disposition for conspecific settlement cues.

Thiyagarajan et al. (2003) elucidated the cyprid energy content at metamorphosis and its implication in growth rate of the early juvenile barnacle *B. amphitrite*. They found a combined effect of early juvenile energy content, temperature and food concentration on the growth until 5 days after metamorphosis. However, 10 days after metamorphosis, the influence of early juvenile energy content on growth was negligible. Furthermore, *in situ* growth of juvenile barnacles has been reported to depend on both independent and interactive effect of cyprid energy reserves (larval nutritional condition) and

habitat (Jarrett and Pechenik, 1997; Jarrett, 2003; Thiagarajan et al., 2005). Studies on physiological condition of *B. amphitrite* (Tremblay et al., 2007) showed that the energy reserves in cypris larvae vary considerably among cohorts. In view of this, settlement success of *B. amphitrite* is related not only to larval supply, but also to the physiological state of the larvae.

## **Sensory mechanisms**

### **Contact and Olfactory chemoreception**

How does a cyprid find its ultimate destination? This has been an often studied question in biofouling research. The process of settlement is divided into three distinct phases, which can be referred as attachment, exploration and fixation. During attachment, a cyprid comes in contact with solid surface and begins crawling over it, but this process is reversible because cyprid can detach and retain its swimming powers if it finds the surface to be unsuitable for settlement and permanent fixation. The exploration process consists of one or more crawling excursions over various surfaces following temporary attachment; intervals between these migrations are occupied by periods of renewed swimming. Fixation, however, is irreversible and determines the ultimate site of the adult; it usually involves an orientation reaction and a cementing process (Crisp, 1955). Studies on settlement behavior and antennular biomechanics in cyprids of *B. amphitrite* (Lagersson and Hoeg, 2002) indicate that the antennular morphology is modified in this species to accommodate “tight direction change” essential for intertidal habitat preference compared to other species of cirripede with simplified settlement behavior. The maneuverable cyprid antennule in *B. amphitrite* consists of four jointed segments, is furnished with numerous chemo- and mechanoreceptor organs, and is supported by the most “complete” nervous system in the cirripede life cycle (Crisp, 1976; Hoeg, 1985; Harrison and Sandeman, 1999).

Okazaki and Shizuri (2000) identified expression of six genes in the cypris larva of *B. amphitrite*, which were absent in conspecific naupliar and adult stages. Thiagarajan and Qian (2008) studied the overall profile of protein expression in the nauplius, swimming cyprid, attached cyprid, and metamorphosed cyprid and revealed that the proteome of a swimming cyprid was distinctly different from the other life stages and expressed more cyprid-specific proteins. Earlier studies indicated that the signal transduction process during metamorphosis is mediated by cAMP (Clare et al., 1995; Rittschof et al., 1986) and calmodulin, which mediates the calcium-associated signal transduction pathway (Yamamoto et al., 1998, 1999). Identification of these signal transduction proteins (adenylate cyclase

and calmodulin) in the proteome of cyprids provide new insight into the mechanisms of cyprid attachment and metamorphosis and needs further validation (Thiyagarajan and Qian, 2008).

Barnacles are obligate cross fertilizers, thus locating their own conspecifics is crucial for their reproductive success (Burke, 1986; Toonen and Pawlik, 1994). Arthropodin or adult extract (AE), a glycoprotein present in the adults is thought to attract conspecifics (Knight-Jones, 1953; Knight-Jones and Crisp, 1953; Crisp and Meadows, 1963). *Lens culinaris* agglutinin-binding sugar chains of the adult extract (AE) have been implicated in the settlement of *B. amphitrite* (Matsumura et al., 1998). Recently, Settlement Inducing Protein Complex (SIPC) of *B. amphitrite* has been identified as a cuticular glycoprotein with sequence similarity to the  $\alpha_2$ -macroglobulin protein family (Dreanno et al., 2006a, b). While exploring some surfaces, cyprids leave behind ‘footprints’ of temporary adhesive (Walker and Yule, 1984; Clare and Matsumura, 2000) which are believed to be secreted by antennular glands that open out onto the antennular attachment disc (Nott and Foster, 1969). The exploratory behavior and subsequent settlement of cyprid when subjected simultaneously to sugars and adult extract (AE) showed that sugar-treated cyprids did not deposit foot-prints in the absence of AE. However, sugar-treated cyprids deposited foot-prints when exposed to adult extract (Khandeparker et al., 2002a). As earlier hypothesized (Yule and Walker, 1987), that sugars in solution adsorb electrostatically through –OH groups to polar groups associated with the cypris temporary adhesive (CTA), the detection of AE and deposition of foot-prints (exploration) was attributed to availability of alternate sites for pheromone reception. It was also suggested (Khandeparker et al., 2002a) that settlement proteins of AE are possibly detected by the receptors on the fourth antennular segment via olfaction. The absence of AE rendered these sites non-functional thus the cyprids responded to sugars in either promotion or inhibition of settlement without further search. Identification of the possible signal transduction pathway that controls the cyprid’s ability to switch the mechanisms of cue perception will be a step ahead.

Implication of biofilm characteristics on settlement have also been assessed by several workers either in pure cultures or natural biofilms (Maki et al., 1988, 1992, 1994; Holmstrom et al., 1992, 1996; Avelin Mary et al., 1993; Wiczorek et al., 1995; O’Connor and Ritchardson, 1996, 1998; Anil and Khandeparker, 1998; Olivier et al., 2000; Khandeparker et al., 2002a, b, 2003, 2005, 2006). Microbial biofilms are generally observed to stimulate settlement of macrofouling organisms (Crisp, 1974). Monospecific bacterial films show varying effects on cyprid attachment (Kirchman et al., 1982; Holmstrom et al., 1992; Avelin Mary et al., 1993; Lau et al., 2003) and the same bacterium may elicit

different responses by different fouling organisms (Maki et al., 1989, 1992). Maki et al. (1990) showed that when adsorbed on different substrata, the same bacterium induces different attachment response by barnacle cyprid.

Neal and Yule (1994) demonstrated that age of the biofilm, rather than surface wettability (which refers to polar dispersive forces at the surface, which are affected by inherent surface chemistry as well as by physical forces (Dahlstrom et al., 2004) as the major factor determining larval adhesion. After adsorbing to the surface, the attached bacteria can alter the substratum characteristics either by changing the surface wettability or by exposing different types of exopolymeric substances (Anil et al., 1997; Khandeparker et al., 2002a, b). These exopolymers and other microbial secretions are involved in settlement of macrofoulers, metamorphosis induction, growth and development of organisms (Maki et al., 1990, 1992, Holmstrom and Kjelleberg, 1994). Bacteria produce surface-bound and soluble chemical cues that either stimulate or inhibit larval settlement of different marine invertebrates (Dobretsov et al., 2006; Hadfield and Scheuer, 1985; Kirchman et al., 1982; Maki et al., 1990; Szewzyk et al., 1991; Maki et al., 1992; Unabia and Hadfield, 1999; Qian et al., 2003). Diatom exopolymers have also been shown to influence the settlement of *B. amphitrite* cyprids whose settlement response varies with different diatom species (Patil and Anil, 2005).

Natural microbial communities found on estuarine and marine substrata, containing a diversity of bacterial species, can stimulate, inhibit, or have no effect on permanent attachment of barnacle cyprid (Strathmann et al., 1981; Maki et al., 1988, 1990). Species composition and age of biofilms influence the settlement success of cyprid of *B. amphitrite* (Qian et al., 2003; Hung et al., 2008). However the relative proportion of inhibitory or stimulatory bacterial strains in biofilms differs with the environmental conditions (Dobretsov et al., 2006), and thus the presence of different bacteria within a biofilm may ultimately determine the community composition of intertidal invertebrates.

Bacteria produce different molecules depending on the nutritional conditions under which they grow, and such differences govern larval settlement (Khandeparker et al., 2006). Surface-bound cues mediate settlement whereas water-borne ones are suggested to be more significant in detecting a substratum covered with a biofilm (Khandeparker et al., 2006). However, lectins can modulate the effect caused by biofilm by interacting with the specific carbohydrate moieties in the bacterial exopolymers and thus play an important role in piloting cyprids to their destination (Khandeparker et al., 2003). The

differing degree of settlement with various combinations of exopolymers with different bacterial strains with or without adult settlement proteins demonstrates the complexities involved. Adult settlement proteins also play a vital role in bacterial modulation of cyprid metamorphosis (Khandeparker et al., 2006). Understanding the synchronization of contradictory signaling molecules from different sources responsible for settlement and metamorphosis needs further validation.

Some bacteria in biofilms are capable of genetic exchange (Fri and Day, 1990). The cell-cell communication between same species of bacteria and perhaps others through N-acyl-L-homoserine lactones (AHL) is a possibility, and same has been demonstrated in natural biofilms (McLean et al., 1997). The characterization of larval receptors that identify EPS that are intimately associated with the cell surface and those released in the free forms would be useful to design probes for such saccharides so that the genes that produce them can be explored (Khandeparker et al., 2003). One unanswered question is the potential concentration or strength of cues that is actually perceived by the planktonic cyprids in nature and further direct them to travel towards the surface? This remains largely unexplored and needs attention.

### **Photo and Auditory reception**

Larval forms of *B. amphitrite* appear to have more complete nervous system than the adults, however functions and sensory mechanisms in all stages of barnacles are not well defined. In particular, the roles of photoreceptors and the possible auditory receptors are not adequately addressed. Doris (1928) reviewing light-receptive organs of *B. eburneus* and *B. balanoides* pointed out that the light perceptive organs present are (1) a median eye in the naupliar (early instar), (2) a median eye and two compound eyes in the metanaupliar (later instar), (3) a median eye and two compound eyes in the cyprid, and (4) two simple eyes in adult. The compound eyes that first appear in the advanced instar of naupliar development remain functional throughout the cyprid stage. However, these compound eyes are resorbed when a cyprid metamorphoses into an adult. During metamorphosis, the median eye divides into two parts that form the simple paired eyes of the adult. Each of the paired eyes in an adult is the morphological equivalent of half of the median eye plus an outer covering, and these are the sole light-perceptive organs of the adult. Sudden changes in light intensity induce adults to close the opercular valves, although very gradual changes do not excite the reaction in *B. balanoides* and *B. eburneus* (Doris, 1928). Visscher and Luce (1928) demonstrated that the peak spectral sensitivity is 530 nm (blue/green)

in cyprids of *B. amphitrite*. It was pointed out that cyprids of *Semibalanus balanoides* may have greater sensitivity at higher wavelengths and exhibit broader spectral response than *B. amphitrite* (Yule and Walker, 1984). Lang et al. (1979) studied the light response of stage VI nauplii of *B. amphitrite* and concluded that compound eyes function over different wavelength spectra and absolute sensitivity thresholds than does the simple median eye.

In an attempt to understand the remodeling of the nauplius eye into the adult ocelli during metamorphosis, comparative study on photoreceptive ability of *B. amphitrite* larval and adult ocelli was made (Takenaka et al., 1993). Their results indicated that ecdysis begins about 8 h after cyprid attachment. Five minutes after ecdysis, juveniles thrust their cirri in and out from the opercular plates, and these juveniles respond to shadows as sharply as adults. Hence, the necessary mechanisms to induce the shadow reflex have already developed as early as the completion of ecdysis. The three components of the naupliar eye begin to remodel to adult ocelli, so some of the ocelli are responsible for shadow reflex. Experiments have also shown that cyprids orient their anterior end away from the source of light suggesting light is an important factor in determining the point of attachment for the cyprid (Visscher and Luce, 1928). The compound eye of larvae is closely associated with a pair of frontal filaments and setae are also thought to function as mechano- and chemoreceptors (Walker, 1974). Setae are located on the antennules (Nott and Foster, 1969; Nott, 1969; Clare and Nott, 1994; Glenner and Hoeg, 1995), thoracic appendages (Glenner and Hoeg, 1995), carapace valves (Walker and Lee, 1976; Jensen et al., 1994; Glenner and Hoeg, 1995) and caudal rami (Walker and Lee, 1976; Glenner and Hoeg, 1995). However, their role in settlement needs further elucidation.

The pair of widely separated compound eyes that are present only in the cyprid stage is related to the importance of visual sensory input needed during settlement, and which obviously cannot be mediated by small spotlike nauplius eye (Barnes et al., 1951; Hallberg and Elofsson, 1983; Harrison and Sandeman, 1999). The primary function of a cyprid is to settle and this is reflected by the fact that the barnacle nervous system is the most “complete” in a cyprid. The cyprid has a well-developed brain and a large investment in cephalic sense organs, whereas the brain is greatly reduced in the naupliar stages and almost completely absent in the adult barnacle (Walley, 1969; Harrison and Sandeman, 1999). Further, neural connections connect between the central nervous system and many of the peripheral sense organs (Harrison and Sandeman, 1999). Thus, the nervous system of the cyprid must sort and process inputs from various sense organs, and coordinate an appropriate behavioral response. However, connections to

the lattice organs and other sensory structures on the carapace remain unexplored. It was concluded that, the large investment in sensory structures, each of which links to a discrete neuropil within the brain, could provide a relatively sophisticated level of neural processing by the nervous system of cyprid (Harrison and Sandeman, 1999).

Cyprids show a photokinetic response to light intensity as well as a phototactic response to light flux. Phototactic behavior is reversible depending upon a larva's past experiences with feeding, pressure and light intensity (Crisp and Ritz, 1973). While studying the phototactic response of Cirripede larvae to light, they found that these larvae are sufficiently sensitive to light to be able to remain within the zone where light would be sufficient for orientation in most coastal waters. They also pointed out that sensitivity decreases in very turbid waters, with strong turbulence carrying the larvae several meters below the surface (Crisp and Ritz, 1973).

Histamine, a ubiquitous aminergic messenger throughout the body, serves as a neurotransmitter in both vertebrates and invertebrates. In particular, the photoreceptors of adult arthropods use histamine, modulating its release to variations in light intensity (Stuart et al., 2002). Their observations on uptake of neurotransmitter  $^3\text{H}$ -histamine into the eyes of barnacle larvae (*B. amphitrite*) showed that any change in the ambient light affects the rhythmic motion of the cirri. In larvae, this motion underlies swimming, whereas in adults it is devoted to feeding. In adult barnacles, rhythmic feeding by the cirri proceeds in the light, but dimming of light decreases rhythmic cirral motion and provokes the retraction of the cirri into the shell (Stuart et al., 2002). Strong evidence from various arthropod species indicate that histamine is synthesized and stored in photoreceptors, undergoes Ca-dependent release, inhibits postsynaptic interneurons by gating Cl channels, and is then recycled. Light depolarizes the photoreceptors, causing histamine release and postsynaptic inhibition; dimming hyperpolarizes the photoreceptors causing a decrease in histamine release and an "off" response in the postsynaptic cell (Stuart et al., 2007).

The relative success of cyprid population to stick to the coastal environment, determines the ultimate success of its population in any given environment. Shanks (1986) observed that cyprids by their position in water column exploit onshore currents which carry them back to the shore. Evidence provided by him, suggests that larvae are transported from offshore plankton to the coastline in surface slicks generated over tidally forced internal waves. Experimental studies in plumes and pipes have

shown that an increase in velocity or boundary shear stress can decrease settlement of barnacles (Koehl, 2007). The capability of larvae to sense such variations can be linked to lattice organs. Ito and Grygier (1990) pointed out that these lattice organs are proprioceptive mechanoreceptors, since they are in close association with the anterior and posterior carapace slits. These organs may have possible role in signaling the extent to which the carapace valves diverge from each other. The presence of a pore or pores also argues for chemosensory function. Laverack and Barrientos (1985) also suggested that lattice organs could act as pressure-sensitive organs. El'fimov (1986) suggested that a pore or pores could allow seawater to reach the subcuticular sensory cells.

A comparison of SEM studies of lattice organs (Jensen et al., 1994) confirmed that they have sensory function and contain paraciliary elements. These lattice organs always occur in 2 + 3 pairing pattern and represent a gradual morphological series from types reminiscent of a sensillum to advanced cuticular porefields found in both the Ascothoracida and in all three orders of the Cirripedia. Moreover, lattice organs occur in similar number and positions in cyprids of species that otherwise differ vastly in settlement substratum, metamorphosis and adult mode of life (e.g. boring Acrothoracica, intertidal balanomorphs, and parasitic Rhizocephala). Thus, lattice organs have the same function throughout the Thecostraca regardless of variations in detailed morphology. This highlights the homology and uniqueness in structure and function of the cypris larva throughout all Cirripedia. Hoeg et al., (1998) demonstrated that lattice organs in cyprids of *Trypetesa lampas* (Acrothoracica) and *Peltogaster paguri* (Rhizocephala) which represent the two main types of lattice organ found in cirripedes, but only minor differences exist in lattice organs at the TEM level and also confirmed that these lattice organs have sensory function as previously suggested by Jensen et al. (1994). *B. amphitrite* carries five pairs of lattice organs (Jensen et al., 1994). Lattice organs have naupliar precursors in the form of setae with pores which indicates that lattice organs function during the pelagic larval phase (Rybakov et al., 2003). This coupled with the observation that there are very considerable morphological differences among cirripede cyprids in terms of both attachment organs and antennular setation, it is possible that such differences will have a role in habitat selection between species (Moyses et al., 1995).

### **Physiological adaptations**

Balanomorphs are primarily sublittoral (73% of species) or intertidal (26% of species) (Foster, 1987). *B. amphitrite* is found from supralittoral to subtidal levels and has euryhaline and eurythermal

survival capabilities. In densely populated marine environments, where free space is a limiting factor, living substrata becomes important for epibiosis (Wahl, 1989; Patil and Anil, 2000). Barnacle epibiosis is also well documented in host organisms, eg. Mussels (Wahl, 1989), sea snakes (Annandale, 1909), sea turtles (Annandale, 1909; Crozier, 1916; Foster, 1978; Hubbs, 1977; Hughes, 1974; Monroe and Limpus, 1979), whales (Clarke, 1966), crabs (Jerde, 1967; Moazzam and Rizvi, 1979), shark (Williams, 1978) and horse shoe crab (Patil and Anil, 2000).

Barnacle zones in rocky shores are precisely defined with regard to tidal height, and are frequently visually obvious (Foster, 1987). Upper limits are largely determined by physical factors associated with the tidal immersion while lower limits are usually set by complex biotic interactions. The barnacle shell provides protection from predation and desiccation while behavioral and physiological adaptations allow barnacles to tolerate reduced feeding time, extreme daily variations in temperature and salinity. Species of barnacles from intertidal habitats are more temperature and desiccation tolerant than subtidal ones, and those that extend their populations to higher shore levels are more tolerant to desiccation than the lower shore ones (Foster, 1987). In this context capabilities differ with the barnacle species. e.g. *Chthamalus* sp. which inhabit high level species has a lower metabolic rate than the other species of barnacles which occupies a lower level in the intertidal habitat (Barnes and Barnes, 1957). When exposed to air during low tide, barnacles respire by taking air into the mantle cavity, and reduce their metabolic activity; thereby satisfying nearly all their aerobic and anaerobic metabolic needs (Grainger and Newell, 1965; Newell, 1979a, b; Davenport, 1976).

Barnacles inhabiting the intertidal area are exposed to extreme physical conditions during low tides (Lewis, 1978; Newell, 1979a, b), and can experience body temperatures that exceed ambient air temperature and regularly experience conditions above their sublethal thermal limits (Helmuth, 1999; Helmuth and Hofmann, 2001; Tomanek and Sanford, 2003). Barnacles can tolerate elevated temperatures by increasing the levels of heat- shock proteins (Hsps), as an induced heat shock response (Berger and Emllet, 2007; Dahlhoff, 2004; Hofmann, 2005; Sanders et al., 1991; Somero, 2002). A recent study in the Pacific Northwest (Berger and Emllet, 2007), indicated that *Balanus glandula* exposed to temperature as high as 34°C for 8.5 h in the laboratory did not show evidence of irreversible protein damage, although higher levels of Hsp70 was observed. Thus, *B. glandula* is well adapted to living in a stressful habitat and may have adequate concentration of heat shock proteins that are required to remediate the protein denaturation.

Intertidal barnacles can survive for long periods of time when they are out of water. Monterosso (1930) noted an occasion when specimens of the high tidal, warm-water, species *Chthamalus depressus* survived at least 119 days in the laboratory at ordinary temperatures. The order of survival of adult *Chthamalus stellatus*, *Balanus balanoides* and *Balanus crenatus* in dry air at unspecified temperatures was correlated with the intertidal distribution of the species (Barnes et al., 1963). One of the adaptation to intertidal existence includes controlled use of the opercular valves when out of water (Barnes and Barnes, 1957, 1964; Crisp and Southward, 1961), which allows intertidal barnacles to use the mantle cavity as a lung for aerobic respiration while minimizing water loss from the surface of the mantle cavity and body (Barnes et al., 1963; Grainger and Newell, 1965; Augenfeld, 1967). Newman (1967) studied water loss in the intertidal barnacle *B. amphitrite* and *B. glandula*, and the low tidal and estuarine *B. improvisus*, and reported that *B. improvisus* lost water at a faster rate than the other 2 species, suggesting that desiccation resistance involved morphological, behavioral and physiological differences.

Respiration as a function of oxygen concentration was studied in two species of intertidal barnacles: *Balanus amphitrite* (DARWIN) and *B. tintinnabulum tintinnabulum* (= *Megabalanus tintinnabulum tintinnabulum*). A comparison of critical oxygen tension in these two species indicated that *Balanus amphitrite* which inhabits oxygen-deficient areas was able to regulate to much lower oxygen concentrations than *B. tintinnabulum tintinnabulum* which inhabits oxygen-rich open intertidal (Prasada and Ganapati, 1968). Oxygen consumption in *B. amphitrite* (adults and nauplii) under normal dissolved oxygen levels is higher than at reduced oxygen levels, suggesting that metabolic rate is reduced during oxidative stress (Desai and Prakash, 2009). The body cells of these species are equipped with an impressive repertoire of antioxidant enzymes, as well as small antioxidant molecules (Yu, 1994) including enzymatic scavengers such as superoxide dismutase (dismutates  $O_2^-$  as well as  $H_2O_2$ ) and catalase (converts  $H_2O_2$  to water). Measurements of antioxidant enzymes such as superoxide dismutase and catalase in *B. amphitrite* collected from different tidal heights, indicated that catalase and superoxide dismutase concentrations in adults increased with increasing levels of desiccation, indicating that the level of antioxidant molecules increase with the tidal height (Desai and Prakash, 2009). This suggests that barnacles have adaptive physiological mechanisms to reduce stress under different levels of exposure.

In a review on various facets of barnacle adhesion (Khandeparker and Anil, 2007), it is discussed that barnacles grown on non-sticky surfaces typically possessed a bell shaped base plate and a thick multilayered adhesive plaque compared to barnacles grown on easy-to-attach surfaces. This peculiar feature was thought to be a result of downward growth of parietal plates and subsequent detachment of the weakly adhered base area (Wiegemann and Watermann, 2003). They also measured the adhesion strength of barnacles during the course of desiccation and found that the shear force required for removing barnacles belonging to different genera varied depending on specific base morphologies (Wiegemann and Watermann, 2004). An evaluation of biofouling and barnacle adhesion data for fouling-release coatings subjected to static immersion at seven different localities around the world also pointed out that there were statistically significant difference in barnacle adhesion strength at different stations (Swain et al., 2000).

Okano et al. (2008a) hypothesized in their study on the molecular basis for the permanent attachment of the cyprid of the barnacle, *Megabalanus rosa* that 1) cyprid cement is composed of several novel proteins that are different from proteins in adult cement, 2) the basic character of the components and the presence of several types of potential protease inhibitors suggest that cyprid cement is well protected from degradation by surrounding bacteria, and 3) cement hardening may be due to cross-linking by lysyl oxidase-like activity. Okano et al. (2008b) also observed that cement release of *Megabalanus rosa* takes place within 1 minute after permanent attachment, and that the extent and appearance of released cement changes with time. They characterized two typical movements just before attachment and called them metronome-like movement and push-up movement. These movements are not clearly separated and sometimes occur simultaneously. The metronome-like movement is dominant, especially within 1 minute after attachment, and the attachment angle sometimes exceed to 100° with time. They hypothesized that this metronome-like movement could be related to dispersion of the released cement, and that the push-up movement could be related to cement mixing and/or cement hardening. The foregoing observations indicate that type of cement produced in cirripedes can change with species, time and situations.

Studying the effect of ocean acidification on the life history of *B. amphitrite* it was observed that despite of enhanced calcification, penetrometry revealed that the central shell wall plates required significantly less force to penetrate than those of individuals raised at higher pH (pH 7.4 v/s pH 8.2). This which indicates that dissolution rapidly weakens wall shells as they grow. At population levels this

result could be important, as barnacles with weakened wall shells are more vulnerable to predators (McDonald et al., 2009). Changes in climate and ocean acidification are important to life processes in the ocean. To understand the effect of climate change, it is imperative to focus on the most sensitive life cycle stages to environmental changes which are often the early developmental and reproductive stages. At these stages environmental requirements are often more specific and acute than at other stages (Thorson, 1950, Kurihara, 2008). Intertidal habitats have served as important models for investigating the effects of climate on species distribution and also monitoring the consequences of climate change for natural ecosystems (Gilmon et al., 2006). Barnacles, which are important members of shallow marine ecosystems (intertidal regions), can serve as candidate organism to understand the effect of climate change. Aldred and Clare (2008) pointed out that the adhesive mechanisms of cyprids have not yet been thoroughly studied using modern analytical methods and should be a focus of future antifouling research. However, variation in the ability of barnacles to attach might also stem from underlying genetic and maternal effects (Holm et al., 2000). The variation in attachment may relate to the rate at which cyprids attain metamorphic competence. Studies integrating the influence of such factors along with expected climatic change features are very much desirable.

### **Acknowledgements**

We are grateful to Dr. S. R. Shetye, Director, National Institute of Oceanography, Goa for his support and encouragement. We also thank Dr. S. S. Sawant, Mr. K. Venkat and other colleagues of MCMRD for their ever willing support. Anonymous reviewer's comments helped us to improve the content and presentation we are indebted to them. This is a NIO contribution #####.

### **References**

- Aldred, N., Clare, A.S., 2008. The adhesive strategies of cyprids and development of barnacle-resistant marine coatings. *Biofouling* 24 (5), 351-363.
- Anger, K., 1987. The  $D_0$  threshold: a critical point in the larval development of decapod crustaceans. *J. Exp. Mar. Biol. Ecol.* 108, 15-30.
- Anil, A.C., Chiba, K., Okamoto, K., Kurokura, K., 1995. Influence of temperature and salinity on the larval development of *Balanus amphitrite*: implications in the fouling ecology. *Mar. Ecol. Prog. Ser.* 118, 159-166.

Anil, A.C., Kurian, J., 1996. Influence of food concentration, temperature and salinity on the larval development of *Balanus amphitrite*. Mar. Biol. 127, 115-124.

Anil, A.C., Khandeparker, L., Mitbavker, S., Wagh, A.B., 1997. Influence of bacterial exopolymers and the adult extract of *Balanus amphitrite* and *Chthamalus* Sp. on cyprid metamorphosis of *Balanus amphitrite*. Emerging Nonmetallic Materials for the Marine Environment. Proceedings of US-Pacific Rim Workshop, Hawaii, USA, 18<sup>th</sup>-20<sup>th</sup> March, pp.1-11.

Anil, A.C., Khandeparkar, R.D.S., 1998. Influence of bacterial exopolymers, conspecific adult extract and salinity on cyprid metamorphosis of *Balanus amphitrite* (Cirripedia: Thoracica). Mar. Ecol. 19 (4), 279-292.

Anil, A.C., Desai, D., Khandeparkar, L., 2001. Larval development and metamorphosis in *Balanus amphitrite* Darwin (Cirripedia: Thoracica): Significance of food concentration, temperature and nucleic acids. J. Exp. Mar. Biol. Ecol. 263 (2), 125-141.

Annandale, N., 1909. The rate of growth in *Conchoderma* and *Lepas*. Records of the Indian Museum 3, 295.

Augenfeld, J.M., 1967. Respiratory metabolism and glycogen storage in barnacles occupying different levels in the intertidal zone. Physiol. Zool. 40, 92-96.

Avelin Mary, S.R., Vitalina Mary, S.R., Rittschof, D., Nagabhushanam, R., 1993. Bacterial-barnacle interaction: potential of using juncellins and antibiotics to alter structure of bacterial communities. J. Chem. Ecol. 19, 2155-2167.

Barnes, H., 1962. Note on variations in the release of nauplii of *Semibalanus balanoides* with special reference to the spring diatom outburst. Crustaceana 4, 118-122.

Barnes, H., Crisp, D.J., 1956. Evidence of self-fertilization in certain species of barnacles. J. Mar. Biol. Assoc. UK 35, 631-639.

Barnes, H., Barnes, M., 1957. Resistance to desiccation in intertidal barnacles. Science NY 126, 358.

Barnes, H., Barnes, M., 1958. The rate of development of *Balanus balanoides* (L.) larvae. Limnol. Oceanogr. 3, 29-32.

Barnes, H., Barnes, M., 1964. Some relations between the habitat, behaviour and metabolism on exposure to air of the high level intertidal cirripede *Chthamalus depressus*. Helgoliinder wiss Meeresunters 10, 19-28.

Barnes, H., Crisp, D.J., Powell, H.T., 1951. Observations on the orientation of some species of barnacles. J. Anim. Ecol. 20, 227-241.

Barnes, H., Barnes, M., Finlayson, D.M., Platigorsky, J., 1963. The effect of desiccation and anaerobic conditions on the behaviour, survival and general metabolism of three common cirripedes. J. Anim. Ecol. 82, 233-252.

- Berger, M.S., Emlet, R.B., 2007. Heat-shock response of the upper intertidal barnacles *Balanus glandula*: Thermal stress and acclimation. *Biol. Bull.* 212, 232-241.
- Bishop, C.D., Erezylimaz, E.F., Flatt, T., Georgiou, C.D., Heyland, A., Hodin, J., Jacobs, M.W., Maslakova, S.A., Pires, A., Reitzel, A.M., Santagata, S., Tanaka, K., Youson, J.H., 2006a. What is metamorphosis? *Integrative and Comparative Biology* 46 (6), 655-661.
- Bishop, C.D., Huggett, M.J., Heyland, A., Hodin, J., Brandhorst, B.P., 2006b. Interspecific variation in metamorphic competence in marine invertebrate: the significance for comparative investigations into the timing of metamorphosis. *Integrative and Comparative Biology* 46 (6), 662-682.
- Blankenship, L. E., Yayanos, A. A., 2005. Universal primers and PCR of gut contents to study marine invertebrate diets. *Mol. Ecol.* 14, 891–899
- Broch, H., 1927. Zoological Results Of The Cambridge Expedition To The Suez Canal, 1924. VII. Report On The Crustacea Cirripedia. *Tr. Zoological Society Of London* 22, 133–137.
- Burke, R.D., 1986. Pheromones and the gregarious settlement of the marine invertebrate larvae. *Bull. Mar. Sci.* 39, 323-331.
- Buskey E. J., Dunton K. H., Parker P. L., 1999. Variations in Stable Carbon Isotope Ratio of the Copepod *Acartia tonsa* During the Onset of the Texas Brown Tide. *Estuaries*, 22 (4), 995-1003.
- Carlton, J.T., Newman, W.A., 2009. Reply to Clare and Hoeg (2008). *Balanus amphitrite* or *Amphibalanus amphitrite*? A note on barnacle nomenclature. *Biofouling* 25 (1), 77-80.
- Charnov, E.L., 1987. Sexuality and hermaphroditism in barnacles: a natural selection approach. In: Southward, A.J. (Ed.), *Crustacean issues 5. Barnacle biology*. Balkema, A. A., Rotterdam, pp. 89–104.
- Clare, A.S., Nott, J.A., 1994. Scanning electron microscopy of the fourth antennular segment of *Balanus amphitrite amphitrite* (Crustacea: Cirripedia). *J. Mar. Biol. Assoc. UK* 74, 967-970.
- Clare, A.S., Thomas, R.F., Rittschof, D., 1995. Evidence for the involvement of cyclic AMP in the pheromonal modulation of barnacle settlement. *J. Exp. Biol.* 198, 655-664.
- Clare, A.S., Matsumura, K., 2000. Nature and perception of barnacle settlement pheromones. *Biofouling* 15, 57-71.
- Clare, A.S. and Hoeg, J.T., 2008. *Balanus amphitrite* or *Amphibalanus amphitrite*? A note on barnacle nomenclature. *The Journal of Bioadhesion and Biofilm Research* 24, 55-57.
- Clarke, R., 1966. The stalked barnacle *Conchoderma* ectoparasitic on whales. *Norsk Hvalfangstid* 55, 153-168.
- Crisp, D.J., 1955. The behavior of barnacle cyprids in response to water movement over a surface. *J. Exp. Biol.* 32, 569-590.

Crisp, D.J., 1974. Factors influencing the settlement of marine invertebrate larvae. In: Grant, P.T., Mackie, A.M. (Eds.), *Chemoreception in marine organisms*. Academic Press, New York, pp. 177-265.

Crisp, D.J., 1976. The role of pelagic larva. In: Davis, P.S. (Ed.), *Perspectives in experimental biology*. Pergamon, New York, pp. 145-155.

Crisp, D.J., Davis, P.A., 1955. Observations in vivo on the breeding of *Elminius modestus* grown on glass slides. *J. Mar. Biol. Assoc. UK* 34, 357–380.

Crisp, D.J., Southward, A.J., 1961. Different types of cirral activity of barnacles. *Phil. Trans. R. Soc.* 248(B), 271-308.

Crisp, D.J., Meadows, P.S., 1963. Adsorbed layers: the stimulus to settlement in barnacles. *Proc. R. Soc. Lond. B.* 158, 364-387.

Crisp, D.J., Ritz, D.A., 1973. Responses of cirripede larvae to light. I. Experiments with white light. *Mar. Biol.* 23, 327-335.

Crozier, W.J., 1916. On a barnacle, *Conchoderma virgatum*, attached to a fish, *Diadon hystrix*. *Am. Nat.* 50, 636-639.

Dahlhoff, E.P., 2004. Biochemical indicators of stress and metabolism: applications for marine ecological studies. *Annu. Rev. Physiol.* 66, 183-207.

Dahlstrom, M., Jonsson H., Jonsson, P.R., Elwing, H., 2004. Surface wettability as a determinant in the settlement of the barnacle *Balanus improvisus* (Darwin). *J. Exp. Mar. Biol. Ecol.* 305, 223-232.

Darwin, C., 1854. A Monograph On The Sub-class Cirripedia. *Tr. Zoological Society Of London* 22, 133–137.

Davenport, J., 1976. A comparative study of some balanomorph barnacles exposed to fluctuating seawater concentrations. *J. Mar. Biol. Assoc. UK* 56, 889–907.

Desai, D.V., Anil, A.C., 2000. Influence of temperature on the starvation threshold of nauplii of barnacle *Balanus amphitrite* (Cirripedia: Thoracica). *Ind. J. Mar. Sci.* 29, 69-72.

Desai, D.V., Anil, A.C., 2002. Comparison of nutritional status of field and laboratory reared *Balanus amphitrite* Darwin (Cirripedia: Thoracica) larvae and implication of starvation. *J. Exp. Mar. Biol. Ecol.* 280, 117-134.

Desai, D.V., Anil, A.C., 2004. The impact of food type, temperature and starvation on larval development of *Balanus amphitrite* Darwin (Cirripedia: Thoracica). *J. Exp. Mar. Biol. Ecol.* 306, 113-137.

Desai, D.V., Anil, A.C., 2005. Recruitment of the barnacle *Balanus amphitrite* in a tropical estuary: implications of environmental perturbation, reproduction and larval ecology. *J. Mar. Biol. Assoc. UK* 85, 909-920.

- Desai, D.V., Anil, A.C., Venkat, K., 2006. Reproduction in *Balanus amphitrite* Darwin (Cirripedia: Thoracica): influence of temperature and food concentration. *Mar. Biol.* 149, 1431-1441.
- Desai, D.V., Prakash, S., 2009. Ecology of *Balanus amphitrite* Darwin (Cirripedia:Thoracica): physiological responses to environmental hypoxia and anoxia. *Mar. Ecol. Prog. Ser.*, *In Press*.
- Dobrestov, S., Dahms, H.U., Qian, P.Y., 2006. Inhibition of biofouling by marine microorganisms and their metabolites. *Biofouling* 22, 43-54.
- Doris, E.F., 1928. The light-receptive organs of certain barnacles. *Biol. Bull.* 54, 534-547.
- Dreanno, C., Matsumura, K., Dohmae, N., Takio, K., Hirota, H., Kirby, R.R., Clare, A.S., 2006a. A novel  $\alpha$ 2-macroglobulin-like protein is the cue to gregarious settlement of the barnacle *Balanus amphitrite*. *Proc. Natl. Acad. Sci. USA* 103(39), 14396-14401.
- Dreanno, C., Kirby, R.R., Clare, A.S., 2006b. Locating the barnacle settlement pheromone: spatial and ontogenetic expression of the settlement-inducing protein complex (SIPC) of *Balanus amphitrite*. *Proc. R. Soc. Lond. Ser. B.* 273, 2721-2728.
- El'fimov, A.S., 1986. Morphology of the carapace of cypris larva of the barnacle *Heteralepas mystacophora*. *Sov. J. Mar. Biol.* 12, 152-156.
- El-Komi, M.M., Kajihara, T., 1991. Breeding and moulting of barnacles under rearing conditions. *Mar. Biol.* 108, 83-89.
- EEl-Sabaawi, R., Dower, J. F., Kainz, M., Mazumder, A., 2009. Characterizing dietary variability and trophic positions of coastal calanoid copepods: insight from stable isotopes and fatty acids. *Mar. Biol.* 156, 225-237
- Flowerdew, M.W., 1985. Indices of genetic identity and distance in three taxa within the *Balanus amphitrite* Darwin complex (Cirripedia, Thoracica). *Crustaceana* 49, 7 – 15.
- Foster, B.A., 1978. The marine fauna of New Zealand: Barnacles (Cirripedia, Thoracica). New Zealand Oceanographic Institute Memoir 69, 1-160.
- Foster, B.A., 1987. Barnacle ecology and adaptation. In: Southward, A.J. (Ed.), *Crustacean Issues* 5, Barnacle biology, Balkema AA, Rotterdam, pp. 113-134.
- Fri, J.C., Day, M.J., 1990. Plasmid transfer in the epilithon. In: Fry J.C., Day M.J. (Eds.), *Bacterial Genetics in the Natural Env't*. Chapman & Hall, London, UK, pp. 55-80.
- Furman, E.R., Yule, A.V., 1990. Self-fertilization in *Balanus improvisus* Darwin. *J. Exp. Mar. Biol. Ecol.* 144, 235-239.
- Gaonkar, C., Anil, A.C., 2009. What do barnacles larvae feed on ? Implications in biofouling ecology. *J. Mar. Biol. Assoc. UK. Inpress*

- Gilmon, S.E., Wethey, D.S., Helmuth, B., 2006. Variation in the sensitivity of organismal body temperature to climate change over local and geographic scale. *PANS* 103, 9560-9565.
- Glenner, H., Hoeg, J.T., 1995. Scanning electron microscopy of cypris larvae of *Balanus amphitrite* (Cirripedia: Thoracica: Balanomorpha). *J. Crustac. Biol.* 15, 523-536.
- Grainger, F., Newell, G.E., 1965. Aerial respiration in *Balanus balanoides*. *J. Mar. Biol. Assoc. UK* 45, 469-479.
- Hadfield, M.G., Scheuer, D., 1985. Evidence for a soluble metamorphic inducer in *Phestilla*: ecological, chemical and biological data. *Bull. Mar. Sci.* 37, 556-566.
- Hadfield, M.G., Carpizo-Ituarte, E., del Carmen, K., Nedved, B.T., 2001. Metamorphic competence, a major adaptive convergence in marine invertebrate larvae. *Am. Zool.* 41, 1123-1131.
- Hallberg, E., Elofsson, R., 1983. The larval Eompond Eyes of Barnacles. *Journal of crustacean biology* 3, 17-24.
- Harding, J.P., 1962. Darwin's type specimens of varieties of *Balanus amphitrite*. *Bull. of British Museum (Natural History)* 9 (7), 274-296.
- Harrison P.J.H., Sandeman, D.C., 1999. Morphology of the nervous system of the barnacle cypris larva (*Balanus amphitrite* Darwin) revealed by light and electron microscopy. *Biol. Bull.* 197, 144-158.
- Helmuth, B., 1999. Thermal biology of rocky intertidal mussels: quantifying body temperature using climatological data. *Ecology* 80, 15-34.
- Helmuth, B., Hofmann, G.E., 2001. Microhabitats, thermal heterogeneity, and patterns of physiological stress in the rocky intertidal zone. *Biol. Bull.* 201, 374-384.
- Henry, D.P., McLaughlin, P.A., 1975. The barnacles of the *Balanus amphitrite* complex (Cirripedia, Thoracica). *Zoologische Verhandelingen Leiden* 141, 1 – 254.
- Hoeg, J.T., 1985. Male cypris settlement in *Clistosaccus paguri* Lilljeborg (Crustacea: cirripedia: Rhizocephala). *J. Exp. Mar. Biol. Ecol.* 89, 221-235.
- Hoeg, J., Hosfeld, B., Jensen, P.G., 1998. TEM studies on the lattice organs of cirripede cypris larvae (Crustacea, Thecostraca, Cirripedia). *Zoomorphology* 118, 195-205.
- Hofmann, G.E., 2005. Patterns of Hsp gene expression in ectothermic marine organisms on small to large biogeographic scales. *Integr. Com. Biol.* 45, 247-255.
- Holm, E.R., McClary Jr., M., Rittschof, D., 2000. Variation in attachment of the barnacle *Balanus amphitrite*: sensation or something else? *Mar. Ecol. Prog. Ser.* 202, 153-162.
- Holmstrom, C., Rittschof, D., Kjelleberg, S., 1992. Inhibition of settlement of larvae of *Balanus amphitrite* and *Ciano intestinalis* by a surface-colonizing marine bacterium. *Appl. Environ. Microbiol.* 58, 2111-2115.

- Holmstrom, C., Kjelleberg, S., 1994. The effect of external biological factors on settlement of marine invertebrate and new antifouling technology. *Biofouling* 8, 147-160.
- Holmstrom, C., James, S., Egan, S., Kjelleberg, S., 1996. Inhibition of common fouling organisms by marine bacterial isolates with special reference to the role of pigmented bacteria. *Biofouling* 10, 251-259.
- Hubbs, C.L., 1977. First record of mating of ridley turtles in California, with notes on commensals, characters, and systematics. *California Fish and Game* 63, 263-267.
- Hughes, G.R., 1974. The sea turtles of South-East Africa, II. Oceanographic Research Institute Investigational Report No. 36. South African Association for Marine Biological Research, Durban, South Africa.
- Hung, O.S., Thiyagarajan, V., Qian, P.Y., 2008. Preferential attachment of barnacle larvae to natural multi-species biofilms: Does surface wettability matter? *J. Exp. Mar. Biol. Ecol.* 361, 36-41.
- Isomura, N., Nishihira, M., 2001. Size variation of planulae and its effect on the life time of planulae in three pocilloporid corals. *Coral Reefs* 20, 309-315.
- Ito, T., Grygier, M.J., 1990. Description and complete larval development of a new species of *Baccalaureus* (Crustacea: Ascothoracida) parasitic in a Zoanthid from Tanabe Bay, Honshu, Japan. *Zool. Sci.* 7, 485-515.
- Jarrett, N.J., 2003. Seasonal variation larval condition and postsettlement performance of the barnacle *Semibalanus balanoides*. *Ecology* 84(2), 384-390.
- Jarrett, N.J., Pechenik, J.A., 1997. Temporal variation in cyprid quality and juvenile growth capacity for an intertidal barnacle. *Ecology* 78 (5), 1262-1265.
- Jensen, P.G., Moyses, J., Hoeg, J.T., Al-yahya, H., 1994. Comparative SEM studies of lattice organs: putative sensory structures on the carapace of larvae from Ascothoracida and Cirripedia (Crustacea Maxillopoda Thecostraca). *Acta. Zool.* 75, 125-142.
- Jerde, C.W., 1967. On the distribution of *Portunus* (Achelous) *affinis* and *Euphyllax dovii* (Decapoda Brachyura, Portunidae) in the eastern tropical Pacific. *Crustaceana* 13, 11-22.
- Khandeparkar, L., Anil, A.C., Raghukumar, S., 2002a. Exploration and metamorphosis in *Balanus amphitrite* Darwin (Cirripedia: Thoracica) cyprids: significance of sugars and adult extract. *J. Exp. Mar. Biol. Ecol.* 281, 77-88.
- Khandeparkar, L., Anil, A.C., Raghukumar, S., 2002b. Factors regulating the production of different inducers in *Pseudomonas aeruginosa* with reference to larval metamorphosis in *B. amphitrite*. *Aquat. Microb. Ecol.* 28(1), 37-54.
- Khandeparkar, L., Anil, A.C., Raghukumar, S., 2003. Barnacle larval destination: piloting possibilities by bacteria and lectin interaction. *J. Exp. Mar. Biol. Ecol.* 289, 1-13.

- Khandeparker, L., Desai, D., Shirayama, Y., 2005. Larval development and post settlement metamorphosis of barnacle *Balanus albicostatus* Pilsbry and serpulid polychaete *Pomatoleios kraussii* Baird: impact of a commonly used antifouling biocide, Irgarol 1051. *Biofouling* 21(3/4), 169-180.
- Kandeparker, L., Anil, A.C., Raghukumar, S., 2006. Relevance of biofilm bacteria in modulating the larval metamorphosis of *Balanus amphitrite*. *Microbiol. Ecol.* 58, 425-438.
- Khandeparker, L., Anil, A.C., 2007. Underwater adhesion: The barnacle way. *International Journal of Adhesion & Adhesives* 27, 165-172.
- Kirchman, D., Graham, S., Reish, D., Mitchell, R., 1982. Bacteria induce settlement and metamorphosis of *Janua (Dexiospira) brasiliensis* Grube (Polychaeta: Spirorbidae). *J. Exp. Mar. Biol. Ecol.* 56, 153-163.
- Knight-Jones, E.W., 1953. Laboratory experiments on gregariousness during settling in *Balanus balanoides* and other barnacles. *J. Exp. Biol.* 30, 584-598.
- Knight-Jones, E.W., Crisp, D.J., 1953. Gregariousness in barnacles in relation to the fouling of ships and to antifouling research. *Nature* 171, 1109-1110.
- Koehl, M.A.R., 2007. Mini review: Hydrodynamics of larval settlement into fouling communities. *Biofouling* 23 (5), 357-368.
- Kurihara, H., 2008. Effects of CO<sub>2</sub>-driven ocean acidification on the early developmental stages of invertebrates. *Mar. Ecol. Prog. Ser.* 373, 275-284.
- Lagersson, N.C., Hoeg, J.T., 2002. Settlement behavior and antennular biomechanics and in cypris larvae of *Balanus amphitrite* (Crustacea: Thecostraca: Cirripedia). *Mar. Biol.* 141, 513-526.
- Lang, W.H., Forward, Jr., R.B., Miller, D.C., 1979. Behavioral responses of *Balanus improvisus* nauplii to light intensity and spectrum. *Biol. Bull.* 157, 166-181.
- Lang, W.H., Marcy, M., 1982. Some effects of early starvation on the survival and development of barnacle nauplii, *Balanus improvisus* (Darwin). *J. Exp. Mar. Biol. Ecol.* 60, 63-70.
- Leslie, H.M., Breck, E.N., Chan, F., Lubchen, J., Menge, B.A., 2005. Barnacle reproductive hospitals linked to nearshore ocean conditions. *Proc. National Academy Sciences, USA* 102, 10534-10539.
- Lau, S.C.K., Thiyagarajan, V., Qian, P.Y., 2003. The bioactivity of bacterial isolates in Hong Kong water for the inhibition of barnacle (*Balanus amphitrite* Darwin) settlement. *J. Exp. Mar. Biol. Ecol.* 282, 43-60.
- Laverack, M.S., Barrientos, Y., 1985. Sensory and other superficial structures in living marine Crustacea. *Transaction of the Royal Society of Edinburgh: Earth Science* 76 (2/3), 123-136.
- Lewis, J.R., 1978. *The ecology of rocky shores*. Hodder and Stoughton, London.

- Love, A.C., 2002. Darwin and Cirripedia Prior to 1846: Exploring the Origins of the Barnacle Research. *Journal of the History of Biology* 35, 251 – 289.
- Lucas, M.I., Walker, G., Holland, D.L., Crisp, D.J., 1979. An energy budget for the free-swimming and metamorphosis larvae of *Balanus balanoides* (Crustacea: Cirripedia). *Mar. Biol.* 55, 221-229.
- Lucas, M.I., Crisp, D.J., 1987. Energy metabolism of eggs during embryogenesis in *Balanus balanoides*. *J. Mar. Biol. Assoc. UK* 67, 27-54.
- Maki, J.S., Rittschof, D., Costlow, J.D., Mitchell, R., 1988. Inhibition of attachment of larval barnacles, *Balanus amphitrite*, by bacterial surface films. *Mar. Biol.* 97, 199-206.
- Maki, J.S., Rittschof, D., Schmidt, A.R., Snyder, A.G., Mitchell, R., 1989. Factors controlling attachment of bryozoan larvae: a comparison of bacterial films and unfilmed surfaces. *Biol. Bull.* 177, 295-302.
- Maki, J.S., Rittschof, D., Samuelsson, M.O., Szewzyk, U., Kjelleberg, S., Costlow, J.D., Mitchell, R., 1990. Effect of marine bacteria and their exopolymers on the attachment of barnacle cypris larvae. *Bull. Mar. Sci.* 46, 499-511.
- Maki, J.S., Rittschof, D., Mitchell, R., 1992. Inhibition of larval barnacle attachment to bacterial films: an investigation of physical properties. *Microb. Ecol.* 23, 97-106.
- Maki, J.S., Yule, A.B., Rittschof, D., Mitchell, R., 1994. The effects of bacterial films on the temporary adhesion and permanent fixation of cypris larvae, *Balanus amphitrite* Darwin. *Biofouling* 8, 121-131.
- Marshall, D.J., Keough, M.J., 2003. Variation in the dispersal potential of non-feeding invertebrate larvae: the desperate larva hypothesis and larval size. *Mar. Ecol. Prog. Ser.* 255, 145-153.
- Marshall, D.J., Pechenik, J.A., Keough, M.J., 2003. Larval activity levels and delayed metamorphosis affect post-larval performance in the colonial ascidian *Diplosoma listerianum*. *Mar. Ecol. Prog. Ser.* 246, 153-162.
- Matsumura, K., Mori, S., Nagano, M., Fusetani, N., 1998. Lentil lectin inhibits adult extract-induced settlement of the barnacle, *Balanus amphitrite*. *J. Exp. Zool.* 280, 213-219.
- McDonald, M.R., McClintock, J.B., Amsler, C.D., Rittschof, D., Angus, R.A., Orihuela, B., Lutostanski, K., 2009. Effects of ocean acidification over the life history of the barnacle *Amphibalanus amphitrite*. *Mar. Ecol. Prog. Ser.* 385, 179-187.
- McEdward, L., 1984. Morphometric analysis of the growth and form of an echinopluteus. *J. Exp. Mar. Biol. Ecol.* 82, 259-287.
- McLean, R.J.C., Whiteley, M., Stickler, D.J., Fuqua, W.C., 1997. Evidence of autoinducer activity in naturally occurring biofilms. *FEMS Microbiol. Lett.* 154, 259-263.

- Moazzam, M., Rizvi, S.H.N., 1979. Some observations of post-cyprid stages and population structure of *Conchoderma virgatum* var. *hunteri* from offshore waters of Pakistan. *Pakistan Journal of Scientific and Industrial Research* 22, 305-307.
- Monroe, R., Limpus, C.J., 1979. Barnacles on turtles in Queensland waters with descriptions of three new species. *Memoris of the Queensland Museum* 19, 197-223.
- Monterosso, B., 1930. Studi cirripedologici. VI. Sul compartimento di *Chthalamus stellatus* in diverse condizioni sperimentali. *Atti. Accad. Naz. Lincei. Re.* 11, 501-505.
- Moyse, J., Hoeg, J.T., Jensen, P.G., Al-Yahya, H.A.D., 1995. Attachment organs in cypris larvae: using scanning electron microscopy. In: Schram, F.R., Hoeg, J.T. (Eds.), *Crustacean Issues 10. New Frontiers in Barnacle Evolution*, Balkema, A.A., Rotterdam, pp. 153-177.
- Neal, A.L., Yule, A.B., 1994. The interaction between *Elminius modestus* Darwin cyprids and biofilms of *Deleya marina* NCMB 1877. *J. Exp. Mar. Biol. Ecol.* 176, 127-139.
- Nejstgaard, J. C., Frischer, M. E., Raule, C. L., Gruebel, R., Kohlberg, K. E., Verity, P. G., 2003. Molecular detection of algal prey in copepod guts and fecal pellets. *Limnol. Oceanogr.: Methods* 1, 29–38
- Nejstgaard, J.C., Tang, K.W., Steinke, M., Dutz, J., Koski, M., Antajan, E., Long, J.D., 2007. Zooplankton grazing on *Phaeocystis*: a quantitative review and future challenges. *Biochemistry* 83, 147-172.
- Newell, R.C., 1979a. *Biology of intertidal animals. Marine Ecological Surveys*, Faversham, United Kingdom.
- Newell, R.C., 1979b. Physical, chemical and biological features of the intertidal zone. In *biology of intertidal animals. Mar. Ecol. Sur. UK* pp. 1.
- Newman, W.A., 1967. On physiology and behavior of estuarine barnacles. *Proc. Mar. Biol. Soc. India, Symp. Crust.*, 1038–1066.
- Nott, J.A., 1969. Settlement of barnacle larvae: surface structure of the antennular attachment disc by scanning electron microscopy. *Mar. Biol.* 2, 248-251.
- Nott, J.A., Foster, B.A., 1969. On the structure of the antennular attachment organ of the cypris larva of *Balanus balanoides* (L.). *Phil. Trans. R. Soc. Lon. (B)* 256, 115-134.
- O'Connor, N.J., Ritchardson, D.L., 1996. Effects of bacterial films on attachment of barnacle (*Balanus improvisus* Darwin) larvae: laboratory and field studies. *J. Exp. Mar. Biol. Ecol.* 206, 69-81.
- O'Connor, N.J., Ritchardson, D.L., 1998. Attachment of barnacle (*Balanus amphitrite* Darwin) larvae: responses to bacterial films and extracellular materials. *J. Exp. Mar. Biol. Ecol.* 226, 115-129.
- Okano, K., Nojiri, Y., Takahashi, Y., Sasaki, S., Ozaki, N., Nogata, Y., Oguro-Okano, M., 2008a. Molecular basis for the permanent attachment of the cyprid of the barnacle, *Megabalanus rosa*. *Current*

topics on barnacle biology. 14<sup>th</sup> international congress on marine corrosion and fouling satellite symposium, Kobe University, Japan, 1<sup>st</sup> August, pp. 10.

Okano, K., Kikuchi, A., Takahashi, Y., Ozaki, N., Nogata, Y., Oguro-Okano, M., 2008b. Behavioral analysis just before and after permanent attachment of the cyprid of the barnacle, *Megabalanus rosa*. Current topics on barnacle biology. 14<sup>th</sup> international congress on marine corrosion and fouling satellite symposium, Kobe University, Japan, 1<sup>st</sup> August, pp. 22.

Okazaki, Y., Shizuri Y., 2000. Structures of six cDNAs expressed specifically at cypris larvae of barnacles, *Balanus amphitrite*. *Gene* 250, 127-135.

Olivier, F., Tremblay, R., Bourget, E., Rittschof, D., 2000. Barnacle settlement: field experiments on the influence of larval supply, tidal level, biofilm quality and age on *Balanus amphitrite* cyprids. *Mar. Ecol. Prog. Ser.* 199, 185-204.

Olson, R.R., Olson, M.H., 1989. Food limitations of planktotrophic invertebrate larvae: does it control recruitment success? *Annu. Rev. Ecol. Syst.* 20, 255-274.

Pandian, T.J., 1969. Yolk utilization in the gastropod *Crepidula fornicate*. *Mar. Biol.* 3, 117-127.

Pandian, T.J., 1970. Ecophysiological studies on the developing eggs and embryos of the European lobster *Homarus gammarus*. *Mar. Biol.* 5, 154-167.

Patil, J.S., Anil, A.C., 2000. Epibiotic community of the horseshoe crab *Tachypleus gigas*. *Mar. Biol.* 136, 699 – 713.

Patil, J.S., Anil, A.C., 2005. Influence of diatom exopolymers and biofilms on metamorphosis in the barnacle *B. amphitrite*. *Mar. Ecol. Prog. Ser.* 301, 231-245.

Patil, J.S., Anil, A.C., 2008. Temporal variation of diatom benthic propagules in a monsoon-influenced tropical estuary. *Contin. Shelf Res.* 28 (17), 2404-2416.

Pechenik, J.A., 1990. Delayed metamorphosis by larvae of benthic invertebrates: does it occur? Is there a price to pay? *Ophelia* 32, 63-95.

Pechenik, J.A., Rittschof, D., Schmidt, A.R., 1993. Influence of delayed metamorphosis on survival and growth of juvenile barnacle *Balanus amphitrite*. *Mar. Biol.* 115, 287-294.

Pechenik, J. A., Wendt, D.E., Jarrett, J.N., 1998. Metamorphosis is not a new beginning. *BioScience* 48, 901-910

Pitombo, F.B., 2004. Phylogenetic analysis of the Balanidae (Cirripedia, Balanomorph). *Zool. Scr.* 33, 261 – 276.

Podolsky, R.D., 1994. Temperature and water viscosity: physiological versus mechanical effects on suspension feeding. *Science* 265, 100-103.

- Prasada Rao, G.V., Ganapati, P.N., 1968. Respiration as a function of oxygen concentration in intertidal barnacles. *Mar. Biol.* 1, 309-310.
- Qian, P.Y., Thiyagarajan, V., Lau, S.C.K., Cheung, S.C.K., 2003. Relationship between bacterial community profile in biofilm and attachment of the acorn barnacle *Balanus amphitrite*. *Aquat. Microb. Ecol.* 33, 225-237.
- Rittschof, D., Maki, J.S., Mitchell, R., Costlow, J.D., 1986. Ion and neuropharmacological studies of barnacle settlement. *Neth. J. Sea. Res.* 20, 269-275.
- Rittschof, D., Clare, A.S., Gerhart, D.J., Avelin, S., Bonaventura, J., 1992. Barnacle *in vitro* assays for biologically active substances: toxicity and settlement inhibition assays using mass cultured *Balanus amphitrite amphitrite* Darwin. *Biofouling* 6, 115-122.
- Rybakov, A.V., Hoeg, J.T., Jensen, P.G., Kolbasov, G.A., 2003. The chemoreceptive lattice organs in cypris larvae developed from naupliar setae. *Zool. Anz.* 242, 1-20.
- Sanders, B.M.C., Hope, C., Pascoe, V.M., Martin, L.S., 1991. Characterization of the stress protein response in two species of *Collisella* limpets with different temperature tolerances. *Physiol. Zool.* 64, 1471-1489.
- Sanford, E., Menge, B.A., 2001. Spatial and temporal variation in barnacle growth in a coastal upwelling system. *Mar. Ecol. Prog. Ser.* 209, 143-157.
- Satuito, C.G., Shimizu, K., Natoyama, K., Yamazaki, M., Fusetani, N., 1996. Age-related settlement success by cyprids of the barnacle *Balanus amphitrite*, with special reference to consumption of cyprid storage protein. *Mar. Biol.* 127, 125-130.
- Scheltema, R.S., Williams, I.P., 1982. Significance of temperature to larval survival and length of development in *Balanus eburneus* (Crustacea: Cirripedia). *Mar. Ecol. Prog. Ser.* 9, 43-49.
- Shanks, A.L., 1986. Tidal periodicity in the daily settlement of intertidal barnacle larvae and an hypothesized mechanism for the cross-shelf transport of cyprids. *Biol. Bull.* 170, 429-440.
- Shimizu, K., Satuito, C.G., Saikawa, W., Fusetani, N., 1996. Larval storage protein of the barnacle, *Balanus amphitrite*: biochemical immunological similarities to vitellin. *J. Exp. Zool.* 276, 87-94.
- Skinner, L.F., Coutinho, R., 2002. Preliminary results on settlement of the barnacles *Tetraclita stalactifera* and *Chthamalus bisinuatus* on a Brazilian tropical rocky shore under upwelling conditions. *Invertebrate Reproduction and Development* 41, 151-156.
- Somero, G.N., 2002. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and cost of living. *Integr. Comp. Biol.* 42, 780-789.
- Southward, A.J., 1975. Intertidal and shallow water Cirripedia of the Caribbean. *Stud Fauna Curacao Carib Islands* 46, 1 – 53.

- Starr, M., Himmelman, J.H., Therriault, J.C., 1991. Coupling of nauplii release in barnacles with phytoplankton blooms: a parallel strategy to that of spawning in urchins and mussels. *J. Plankt. Res.* 13 (3), 561-571.
- Stone, C.J., 1986. The effects of variations in diet, temperature and salinity on the development of cirripede nauplii. Ph. D. thesis, University of Wales, UK, pp. 340.
- Stone, C.J., 1988. Test of sequential feeding regimes for larvae of *Elminius modestus* Darwin (Cirripedia: Balanomorpha). *J. Exp. Mar. Biol. Ecol.* 115, 41-51.
- Storch, V.K., Anger, K., 1983. Influence of starvation and feeding on the hepatopancreas of larval *Hyas araneus* (Decapoda, Majidae). *Helgolander Meeresunters* 36, 67-75.
- Strathmann, R.R., 1971. The feeding behavior of planktotrophic echinoderm larvae: mechanisms, regulation, and rates of suspension-feeding. *J. Exp. Mar. Biol. Ecol.* 6, 109-160.
- Strathmann, R.R., 1987. Larval feeding. In: Giese, A.C., Pearse, J.S., Pearse, V.B. (Eds.), *Reproduction of marine invertebrates*. Blackwell scientific publication, The boxwood Press, California, pp. 465-550.
- Strathmann, R.R., Jahn, T.L., Fonseca, J.R.C., 1972. Suspension feeding by marine invertebrate larvae: clearance of particles from suspension by ciliated bands of a rotifer, pluteus, and trochophore. *Biol. Bull. (Woods Hole, Mas.)* 142, 505-519.
- Strathmann, R.R., Bonar, D., 1976. Ciliary feeding of torneria larvae of *Ptychodera flava* (Hemichordata: Enteropneusta). *Mar. Biol.* 34, 505-519.
- Strathmann, R.R., Branscomb, E.S., Vedder, K., 1981. Fatal errors in set as a cost of dispersal and the influence of intertidal flora on set of barnacles. *Oecologia* 48, 13-18.
- Stuart, A.E., Mekeel, H.E., Kempter, E., 2002. Uptake of the neurotransmitter histamine into the eyes of larvae of the barnacle (*Balanus amphitrite*). *Biol. Bull.* 202, 53-60.
- Stuart, A.E., Borycz, J., Meinertzhagen, I.A., 2007. The dynamics of signaling at the histaminergic photoreceptor synapse of arthropods. *Progress in Neurobiology* 82, 202-227.
- Swain, G., Anil, A.C., Baier, R.E., Chia, F., Conte, E., Cook, A., Hadfield, M., Haslbeck, E., Holm, E., Kavanagh, C., Kohrs, D., Kovach, B., Lee, C., Mazzella, L., Meyer, A.E., Qian, P., Sawant, S.S., Schultz, M., Sigurdsson, J., Smith, C., Soo, L., Terlizzi, A., Wagh, A., Zimmerman, R., Zupo, V., 2000. Biofouling and barnacle adhesion data for fouling-release coatings subjected to static immersion at seven marine sites. *Biofouling* 16, 331-344.
- Szewzyk, U., Holmström, C., Wrangstadh, M., Samuelsson, M.O., Maki, J.S., Kjelleberg, S., 1991. Relevance of the exopolysaccharide of marine *Pseudomonas* sp. strain S9 for the attachment of *Ciona intestinalis* larvae. *Mar. Ecol. Prog. Ser.* 75, 259-265.
- Takenaka, M., Suzuki, A., Yamamoto, T., Yamamoto, M., Yoshida, M., 1993. Remodeling of the nauplius eye into the adult ocelli during metamorphosis of the barnacle *Balanus amphitrite hawaiiensis*. *Dev. Growth Differ.* 35, 245-255.

- Tamelander T, Søreide, J. E., Hop, H., Carroll, M. L., 2006. Fractionation of stable isotopes in the Arctic marine copepod *Calanus glacialis*: Effects on the isotopic composition of marine particulate organic matter. *J Expt Mar Biol Ecol*, 333(2), 231-240.
- Thiyagarajan, V., Harder, T., Qian, P.Y., 2002. Relationship between cyprid energy reserves and metamorphosis in the barnacle *Balanus amphitrite* Darwin (Cirripedia; Thoracica). *J. Exp. Mar. Biol. Ecol.* 280, 79-93.
- Thiyagarajan, V., Harder, T., Qian, P.Y., 2003. Effects of TAG/DNA ratio and age of cyprids on post-metamorphic growth and survival in the barnacle *Balanus amphitrite*. *J. Mar. Biol. Assoc. U.K.* 83, 83-88.
- Thiyagarajan, V., Hung, O.S., Chiu, J.M.Y., Wu, R.S.S., Qian, P.Y., 2005. Growth and survival of juvenile barnacle *Balanus amphitrite*: interactive effects of cyprid energy reserve and habitat. *Mar. Ecol. Prog. Ser.* 299, 299-237.
- Thiyagarajan, V., Qian P.Y., 2008. Proteomic analysis of larvae during development, attachment and metamorphosis in the fouling barnacle, *Balanus amphitrite*. *Proteomics* 8, 3164-3172.
- Thorson, G., 1950. Reproduction and larval ecology of marine bottom invertebrates. *Biology Reviews* 25, 1-45.
- Toonen, R., Pawlik, J., 1994. Foundations of gregariousness. *Nature* 370, 511-512.
- Toonem, R.J., Pawlik, J.R., 2001. Settlement of the gregarious tube worm *Hydroides dianthus* (Polychaeta: Serpulidae). II. Testing the “desperate larva” hypothesis. *Mar. Ecol. Prog. Ser.* 224, 115-131.
- Tomanek, L., Sanford, E., 2003. Heat-shock protein 70 (Hsp70) as a biochemical stress indicator: an experimental field test in two congeneric intertidal gastropods (genus: Tegula). *Biol. Bull.* 205, 276-284.
- Tremblay, R., Olivier, F., Bourget, E., Rittschof, D., 2007. Physiological condition of *Balanus amphitrite* cyprid larvae determines habitat selection success. *Mar. Ecol. Prog. Ser.* 340, 1-8.
- Turner, J.T., Levinsen, H., Nielsen, T.G., Hansen, B.W., 2001. Zooplankton feeding ecology: grazing on phytoplankton and predation on protozoans by copepod and barnacle nauplii in Disko Bay, West Greenland, *Mar. Ecol. Prog. Ser.* 221, 209-219.
- Unabia, C.R.C., Hadfield, M.G., 1999. Role of bacteria in larval settlement and metamorphosis of the polychaete *Hydroides elegans*. *Mar. Biol.* 133, 55-64.
- Utinomi, H., 1967. Comments on some new and already known cirripedes with amended taxa, with special reference to the parietal structure. *Publ. Setp. Mar. Bio. Lab.* 15, 199 – 237.
- Vargas, C.A., Manriquez, P.H., Navarrete, S.A., 2006. Feeding by larvae of intertidal invertebrates: assessing their position in pelagic food webs. *Ecology* 87 (2), 444-457.
- Vestheim, H., Edvardsen, B., Kaartvedt, S., 2005. Assessing feeding of a carnivorous copepod using species-specific PCR. *Mar. Biol.* 147, 381–385

- Visscher, J.P., Luce, R.H., 1928. Reactions of the cyprid larvae of barnacles to light with special reference to spectral colors. *Biol. Bull.* 54, 336-350.
- Wahl, M., 1989. Marine epibiosis. I. Fouling and antifouling: some basic aspects. *Mar. Ecol. Prog. Ser.* 58, 175-189
- Walker, G., 1974. The fine structure of the frontal filament complex of barnacle larvae (Crustacea: Cirripedia). *Cell Tissue Res.* 152, 449-465.
- Walker, G., Lee, V.E., 1976. Surface structures and sense organs of the cypris larva of *Balanus balanoides* as seen by scanning and transmission electron microscopy. *J. Zool. (Lond.)*. 178, 161-172.
- Walker, G., Yule, A.B., 1984. Temporary adhesion of the barnacle cyprid: the existence of an antennular adhesive secretion. *J. Mar. Biol. Assoc. UK* 64, 679-686.
- Walley, L.J., 1969. Studies on the larval structure and metamorphosis of *Balanus balanoides* (L.). *Philos. Trans. R. Soc. Lond. B* 256, 237-279.
- Wieczorek, S.K., Clare, A.S., Todd, C.D., 1995. Inhibitory and facilitory effects of microbial films on settlement of *Balanus amphitrite amphitrite* larvae. *Mar. Ecol. Prog. Ser.* 119, 221-228.
- Wiegemann, M., Watermann, B., 2003. Peculiarities of barnacle adhesive cured on non-stick surfaces. *J. Adhesion Sci. Technol.* 17, 1957-1977.
- Wiegemann, M., Watermann, B., 2004. The impact of desiccation on the adhesion of barnacles attached to non-stick coatings. *Biofouling* 20, 25-45.
- Williams, E.H., Jr., 1978. *Conchoderma virgatum* (Spengler) (Cirripedia, Thoracica) in association with *Dinemoura latifolia* (Steenstrup & Lutken) (Copepoda, Caligidea), a parasite of the shortfin mako, *Isurus oxyrinchus* (Rafinesque) (Pisces, Chondrichthyes). *Crustaceana* 34, 109-110.
- Yu, B.P., 1994. Cellular defenses against damage from ROS. *Physio. Rev.* 74, 139-162.
- Yamamoto, H., Tachibana, A., Saikawa, W., Nagano, M., Matsumura, K., Fusetani, N., 1998. Effects of calmodulin inhibitors on cyprid larvae of the barnacle, *Balanus amphitrite*. *J. Exp. Zool.* 280, 8-17.
- Yamamoto, H., Shimizu, K., Tachibana, A., Fusetani N., 1999. Roles of dopamine and serotonin in larval attachment of the barnacle, *Balanus amphitrite*. *Comp. Physiol. Biochem.* 284, 746-758.
- Yule, A.B., 1984. The effect of temperature on the swimming activity of barnacle nauplii. *Mar. Biol. Lett.* 5, 1-11.
- Yule, A.B., Walker, G., 1984. The temporary adhesion of barnacle cyprids: effects of some differing surface characteristics. *J. Mar. Biol. Assoc. UK* 64, 429-439.
- Yule, A.B., Walker, G., 1987. Adhesion in barnacles. In: Southward, A.J. (Ed.), *Crustacean Issues* 5, Barnacle biology, Balkema AA, Rotterdam, pp. 389-402.