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**Captive breeding of the caramote prawn (*Penaeus kerathurus*) for restocking purposes
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Tesi di dottorato di:
Dr. Matteo Ciani

Coordinatore del corso:
Prof.ssa Roberta Cimmaruta

Firma.....

Tutor:
Prof. Giuseppe Nascetti

Firma.....

Co-Tutor:
Dr. Claudio Carere

Firma.....

Data della discussione

“Non chi comincia, ma quel che persevera.”

LEONARDO DA VINCI (1452-1519)

Summary

Increasing fishing efforts over the last 50 years as well as unsustainable fishing practices are pushing many fish and invertebrate stocks to collapse. As a consequence, different global actions were undertaken with the aim of restoring these stocks and achieving healthy and productive oceans. One of the key actions in achieving these goals, at least for certain species, is the now consolidated common practice of “restocking”, i.e. releasing hatchery produced juveniles into the wild to restore the spawning biomass. The strong commercial interest linked to aquaculture increased the knowledge of the early larval stages of many fish and invertebrate species contributing to the development of restocking projects. Thanks to the sessile or sedentary nature of many invertebrates, the latter are likely to be more amenable to restocking than finfish. However, little is still known about many aspects of prawn developmental biology, and its breeding in captivity. In this work we focus on the caramote prawn (*Penaeus kerathurus*), a primary target of small-scale fishery because of its high commercial value in the Mediterranean region and particularly in Italy. Intense exploitation rate in last years depleted *P. kerathurus* stocks, thus species-specific initiatives toward sustainable management of this resource should be implemented in order to strengthen wild stocks and thereby increase catches. Due to the long period required to reach market size and the low resistance to low temperatures, the captive breeding of this prawn is not economically convenient for commercial purposes and information about the optimal diet and food regime of larvae and post-larvae are still scanty. In fact, a crucial step for successful restocking actions is the optimization of diet for larval and post-larval growth. During the larval planktonic phase three species of microalgae, alone and in combination, *Isochrysis galbana*, *Nannochloropsis oculata*, and *Chaetoceros calcitrans*, were tested. These algae have different fatty acid profiles and we aimed at identifying the best diet in terms of development and survival. Diet assessment was continued during the benthonic phase, when drastic changes occur. Post-larvae were fed for a period of 8-weeks using four different diets (frozen mussels, frozen mysis, artificial wet mash, dry pellet) aiming at identifying a diet which could allow a rapid growth, decreasing the costs associated with a longer maintenance of post-larvae in the hatchery, and

reduce the natural mortality related to prawn size for a better restocking efficacy. Results showed that larvae fed algae mix performed as well or slightly better than those fed single *C. calcitrans* or *N. oculata* diets in both development and survival, while the worst performance was with *I. galbana* diet. As far as the growth of subsequent post-larval stage is concerned, the dry pellet diet containing fish meal, seafood meal, wheat meal, astaxanthin, zeaxanthin, lecithin and vitamins with almost 40% in crude protein, was a good solution for a captive breeding aiming at restocking programs. Finally, a first restocking trial of about 10,000 carapide prawn post-larvae was carried out in 2015 along the coastal area of northern Lazio, Italy, in a SCI zone (Site of Community Importance). In this area, within Natura 2000 Network, were previously allocated on the sea bottom 550 deterrent structures to reduce significantly illegal trawling activities. After a short acclimatisation period, post-larvae were released by scuba divers directly on a sandy-muddy bottom. Future survey will be necessary, especially in cooperation with the local small-scale gillnets fisheries, to evaluate the potential aid given to the carapide prawn stock by restocking programs.

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1 Introduction

1.1 Towards an ecological approach

The recent “Agenda 2030” is a collection of 17 global goals (Sustainable Development Goals-SDGs) set by the United Nations. Many countries will mobilize efforts to achieve them in the near future. In particular, SDG 14 (Conserve and sustainably use the oceans, seas and marine resources for sustainable development) aims at sustainably managing and protecting marine and coastal ecosystems to avoid significant adverse impacts by strengthening their resilience, and taking action for their restoration. Moreover, it implements science-based management plans in order to restore fish stocks in the shortest time feasible, at least to levels that can produce maximum sustainable yield as determined by their biological characteristics. The first steps in this direction have been undertaken since 1979 Berne Convention, followed respectively by the Rio de Janeiro Convention on Biological Diversity in 1992 and the Johannesburg World Summit on Sustainable Development (2002), each one of them with the aim to solve problems deriving from overfishing through government actions. The key challenges facing degraded fisheries are the restoration of spawning biomass to more productive levels and the reparation of damaged fish nursery habitats. In the framework of the approach adopted on a global scale, it is clear that in recent years the focus on realising even more effective restocking or stock enhancement strategies has been growing. Restocking involves the release of cultured juveniles into the wild to restore the spawning biomass of a severely depleted fishery to a level from which it can once again provide regular substantial yields. Stock enhancement, on the other hand, is designed to increase the productivity of an operational fishery by overcoming recruitment limitation (Munro and Bell 1997; Doherty 1999; Bell et al. 2005; Lorenzen 2005). Restocking and stock enhancement should be sequential for any given species; severely over-fished population could be restored by restocking and then managed for optimum productivity through stock enhancement.

Synergies between aquaculture and fishery management through the release of cultured juveniles have been recognised for at least 30 years. This applies particularly to Japan, where hatchery-reared juveniles were released to augment wild stock of 90 coastal fish and invertebrate species (Honma 1993; Imamura 1999). The efforts in Japan and in other countries provided important lessons about the potential use of restocking and stock enhancement and implementation of release programmes. Large-scale release of many species has only been made possible through investments in aquaculture, although where there are severe limits to juveniles production because of high costs and restriction of capacity, the use of hatchery-reared juveniles should be considered only for restocking. Although stock enhancement of some finfish has been successful at the scale of large bays (Kitada and Kishino 2006), the efficiency of non-salmonid marine finfish restocking actions has been questioned (Blaxter 2000). Doubt arises because of the trivial contribution of hatchery releases relative to natural reproduction in large population of demersal finfish. On the other hand, the inshore distribution of many sessile or sedentary marine invertebrates, which often promotes meta-population or multiple, self-recruiting populations at surprising small scales (Caddy and Defeo 2003; Bell et al. 2005), sets the stage for hatchery releases to have a significant effect on population size.

1.2 Penaeids and aquaculture

As early as 80 years ago, Hudinaga successfully spawned and reared *Penaeus japonicus* larvae to the mysis stage (Hudinaga 1935). In 1942, one of his papers entitled "Reproduction, development and rearing of *Penaeus japonicus* Bate" was published and became the primary base for prawn research. Unfortunately, World War II interrupted further developments for over 10 years. It was not until the late 50s that several US scientists became highly interested in penaeid hatchery work. In collaboration with Hudinaga, two species of American penaeids, the white shrimp, *P. setiferus*, and the brown shrimp, *P. aztecus*, were spawned and successfully reared in 1963 (Hanson and Goodwin 1977). However, two publications of Hudinaga and Kittaka, namely "Studies on food and growth of larval

stages of a prawn, *Penaeus japonicus*, with reference to the application to practical mass culture" in 1966 and "The large scale production of the young kuruma prawn, *Penaeus japonicus* Bate" in 1967, contributed to the breakthrough in the mass production of penaeid prawns. These two publications set the bases of prawn culture industry. Twenty-four *Penaeus* species and seven *Metapenaeus* species can now be partially or fully artificially propagated. Among these 31 species, on 9 of them, *P. aztecus*, *P. duorarum*, *P. japonicus*, *P. monodon*, *P. orientalis*, *P. setiferus*, *P. stylirostris*, *P. vannamei* and *Metapenaeus ensis*, practical propagation is carried out a commercial scale.

1.3 Prawn restocking plans

In the 70s in Japan, the Japan Fishery Agency located the "Seto Inland Sea" area as a sampling coastal area in which to promote the increase of natural lots thanks to a coastal fishing managerial plan of the local penaeid prawn, *Penaeus japonicus*, through the restocking with lots produced in laboratory. Thanks to restocking activities, the production of prawns from catch at sea increased and ranged from a minimum of 2,000 to 3,700 t with an average of around 2,500 t, with variations (Fushimi 2005; Hamasaki and Kitada 2006). Many other restocking activities testify their positive aspects: in Japan, in the Tokushima bay, where 2 million 50mm young specimens were released, a catch with trammel net of 10 t of prawns (300,000 specimens) was obtained, corresponding to 15% of the restocked specimens (Lumare et al. 1984); in the Kagoshima area, where fishermen caught about 3 t of products/year in the sea, thanks to the restocking activities carried out continuously for 10 years, there was an increase in the fishery production of 13 t/year (Lumare et al. 1984); in China the penaeid prawn *Penaeus chinensis* was lowered for with the purpose of the restocking between the end of the 80s and the beginning of the 90s in a quantity of about 1 billion of prawns per year (Jia and Chen 2001), and also in Kuwait (Farmer 1981) and in Philippines, where the high fishing pressure on the prawns' natural populations was creating critical situations for the artisanal fishery. In the 80s and the beginning of the 90s, restocking activities were also carried out with the Japanese penaeid prawn in Italy in confined marine areas (coastal lagoons, ponds, etc.) by the CNR of Lesina (Lumare 1984,

1986). The results were varying and subordinate to the chemical-physical conditions of the water mass, the alieutic composition according to the pressure of natural predation, trophism, restocking size and other different factors. In good condition habitats, with poor natural production and high trophic regime (Lesina lagoon, Varano lagoon), results highlighted recoveries 23.6 – 32.5% more than the restocked specimens, up to 46.0%, with also the capture of many tonnes of market-size product (Lumare e Hiramatsu 1982; Lumare 1987, 1988, 1990).

Therefore, during these years, prawns breeding models were developed especially for species with a good growth rate, excellent resistance and a high market value. Indeed, there is a minor knowledge about breeding techniques of species like the caramote prawn (*Penaeus kerathurus*), for which juvenile or adult farming is not economically convenient for commercial purposes because of slow growth rates and many limitations associated with hatchery management (Lumare 2001). Despite that, the caramote prawn is one of the primary targets of small-scale fishery that operates with standing nets because of its high commercial value (it can reach very high quotations ranging between 35,00 and 60,00 €/kg) in some Mediterranean coastal regions and particularly in Italy.

2 Captive breeding critical points

Linked to prawn biology, at least two fundamental moments of the production process can be distinguished and each one of them requires specific knowledge and a consequent planning of management interventions. Indeed, a hatchery should have provisions for broodstock maintenance and induced maturation, and after spawning and hatching, for larval and post-larval rearing (phytoplankton and zooplankton live feed culture, fresh and frozen food, pellets).

2.1 Sexual maturation

In captive conditions, sexual maturation can be achieved in laboratory tanks in which the chemical-physical conditions of the environment are kept under control, acting on the biological processes of the species. Generally, it is possible to distinguish three levels on which to act, separately or in combination, to stimulate ovarian maturation in prawns: endocrine, environmental and dietary levels.

2.1.1 Endocrine factors

In female *Parapeneopsis stylzfera*, that had been injected with the hormone progesterone, a higher gonado-somatic index (GSI) and a greater diameter of eggs were observed compared to specimens that were treated with ethanol or were untreated (Joshi 1980). Also studies on *Metapenaeus ensis* and *Cherax albidus* showed the positive effect of progesterone on sexual maturation and reproduction (Yano 1985, Coccia et al. 2010).

Another method to stimulate the reproductive endocrine system is the removal of one (unilateral) or both (bilateral) eyestalks that causes a lowering of gonad-inhibitory hormone (GIH) (Panouse 1943, 1947). Indeed, two antagonist hormones regulate ovary development in crustaceans, the GIH from the X-organ sinux gland complex located in the eyestalk (Treerattrakool et al. 2008, Yan et al. 2014), and the gonad-stimulating hormone (GSH) in the brain and thoracic ganglion (Wongprasert et al. 2006). This hormone is released in the non-breeding season and is absent or present only in low

concentrations during the breeding season (De Kleijn and VanHerp 1995). Eyestalk ablation and consequent low level of GIH leads to increased total egg production and percentage of females that will participate in reproduction. This system is now widely used in commercial farms on many prawn species. To reduce pain, it is preferable to keep the animals in water, at an average temperature lower than the ambient temperature. Eyestalk ablation is carried out by any one of the following methods: cutting, incision and pinching or electrocauterisation. Cutting is performed by complete removal of eye with eyestalk using a sharp instrument, while incision and pinching is carried out by making an incision on the eyeball using a sharp blade and squeezing out the contents of the eyeball. Electrocauterisation is performed by cutting the eyestalk using a cautery apparatus, and is the best method of eyestalk ablation, since it has the advantage of minimising stress. With regard to the experiences carried out in Italy in the case of *P. kerathurus* ablation, the mortality rate was relatively high, around 18%, while no mortality was reported in *P. japonicus* (Lumare 1979).

2.1.2 Environment

A reduction in the intensity of natural light (10-60%) accelerates sexual maturation in *P. merguiensis* (Aquacop 1983, Hoang et al. 2002) and *P. monodon* (Emmerson 1983; Hillier 1984). Light quality also has a considerable influence on sexual maturation; blue and white light radiations did not determine the ovogenesis in *P. duorarum*, after a unilateral eyestalk ablation (Caillonet 1972); on the other hand, red radiation seems to have a positive effect on *P. monodon* sexual maturation (Pudadera and Primavera 1981). The alternation of light and dark (photoperiodism), with a larger photophase, exerts a positive effect on gonad maturation on *P. japonicus* (Laubier-Bonichon and Laubier 1979) and *P. kerathurus* (Lumare 1979), while it does not seem to exert any effect on *P. duorarum* (Caillonet 1972) and on *P. monodon* (Beard and Wickins 1980). Photoperiod did not seem to have a definite influence on sexual maturation on prawns in tropical areas (*P. paulensis*, *P. orientalis*), where there were no significant difference in the number of hours of light and darkness (Spring 1984).

Temperature is another important parameter in Peneids reproduction. For tropical species, an increase of water temperature from 26 to 32 °C seems necessary to activate sexual maturation, whereas for temperate zone species, such as *P. japonicus* and *P. orientalis*, a lower range of values is sufficient (16 - 25 °C). Kanazawa (1982a) obtained repeated captive breeding of *P. japonicus* working between a range of 24 °C and 32 °C, a better result than Laubier-Bonichon and Laubier (1979) who operated at 20 °C. Similar results were also obtained with *P. esculentus*.

Also salinity is correlated to sexual maturation (Chengsong et al. 2014). Indeed, *P. indicus* (Primavera 1984), *P. monodon* (Ruangpanit et al. 1985) and *P. japonicus* do not mature at salinity above 40‰. Finally, pH is considered as one of the most important parameters in sexual maturation control. In *P. indicus* the ovaries were observed to undergo an inversion of development when pH falls from 8.2 to 7.2 (Muthu et al. 1984). Optimal conditions of sexual maturation and reproduction were achieved with a pH of 8.2.

2.1.3 Diet

Diet quality plays an important role in peneids sexual maturation. Lipids and carbohydrates are of fundamental importance on reproductive physiology, as they are related to the ovogenesis in *P. japonicus* (Tashima and Kanazawa 1983) and *P. aztecus* (Chamberlain and Lawrence 1983). Some polyunsaturated fatty acids are particularly important for *P. Monodon* sexual maturation (Spring 1984). A similar situation has also been found for *P. setifèrus*, *P. stylirostris* and *P. vannamei* (Middleditch et al. 1979, 1980). With regards to the feed used to promote sexual maturation in breeders, the most common products are mollusks, mussels, clams, oysters and squid (Shailender et al. 2012; Chimsung 2014). In some cases, pellets are also used as food supplements (Muthu and Laximinarayana 1982) as well as polychaetes (Lawrence et al. 1980).

2.2 Penaeids prawn nutritional requirements

Provision of supplementary feeding to prawns and their larvae is essential for successful hatchery management. Maintenance and production of live feeds escalates the overall cost of seed production. Alternatively, formulated feeds, which are cheaper, decrease the cost of seed production appreciably but are unlikely an alternative to live feeds. Hence, these feeds are supplementary to live feeds only. These formulated feeds have all the essential ingredients such as proteins, lipids (fats), carbohydrates, vitamins, minerals, enzymes and a variety of other components, which add to the flavour and enable to enhance growth and survival. The nutritional requirements of prawns at different stages vary greatly from the larval to the adult stage. Since animals switch their feeding habits frequently during the larval stages, the protocol of larval feeds changes; other factors like particle size, feeding rate and composition play a significant role in the development of a nutritive feed. A number of larval feeds have come out in the market and some have shown reasonably good acceptance in prawn hatcheries. However, research and development programs are going on for better products using modern biotechnological innovations.

Much research was carried out to evaluate the requirements in different species under different conditions. A literature review has clearly shown that feed requirement varied with age of the candidate species. In early stages, the requirement of protein ranged from 50-53% and declined with age to 30-35% in adult breeders. Moreover, as a standard scale to evaluate the nutritional value of feed protein, Phillips and Brockway (1956) suggested that the feed having an essential amino acid composition similar to that of the target species would be the best. Crustaceans, like fish, have 10 amino acids (AA) that are essential for growth: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Hew and Cuzon 1982; Kanazawa 1982b; Guillaume 1989; Chen 1993; Teshima et al. 1997; Ehigiator and Oterai 2012). Proteins can be used effectively for energy production. To maximise the efficiency of protein utilization for somatic growth, it is imperative to balance the supply of protein and non-protein energy in the diet. For instance, if insufficient carbohydrate and/or lipid are present in the diet, crustaceans will use

protein for energy instead of growth. Excess dietary protein may also lead to energy loss and reduction in growth due to the diversion of energy toward the deamination of excess AA.

The lipid requirement ranges from 6 to 8% but never exceeds beyond 13%. In adults, however, the requirements goes up to 10-11% as most part of this is mobilized for gonadal development. Conklin et al. (1980) found that the addition of 6–8% soy lecithin in casein-based diets decreased the incidence of moult death. Cholesterol is one of the most expensive ingredients in artificial diets, with an addition of 0.2% cholesterol representing around 10% of the total cost (Smith et al. 2001). Therefore, it is important to optimise the requirements to allow least cost formulation of crustacean diets. Essential fatty acids (EFA) are those that must be supplied in the diet for normal growth and development. In crustaceans, five FA have been identified as essential for growth and survival: linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3), linoleic acid (LA, 18:2n-6), docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6).

Carbohydrate content was generally considered at uniform level ranging from 15-25% but not above 30%. Kanazawa (1982b) demonstrated that simple sugars, such as glucose and galactose, are poorly utilised by some crustaceans and that the inclusion of more than 10% of these monosaccharides in a formulated diet inhibited the growth of marine prawn, *P. japonicus*. In contrast, disaccharides (sucrose and maltose) and polysaccharides (starch and dextrin) have a high nutritional value, are readily digestible and used for energy production, thus sparing protein in *P. japonicus* (Kanazawa 1982b). Bautista (1986) and Guillaume (1989) suggested that a dietary carbohydrate level of 15–20% is optimal for *Penaeus japonicus* and *P. monodon*.

Little is known about the vitamin and mineral requirements of crustaceans. Vitamin and mineral premixes based on terrestrial or fish requirements are routinely added to crustacean feeds, and due to lack of knowledge, vitamins may often be supplied in excess of requirements (Conklin et al. 1991). Excessive levels of certain vitamins may be detrimental to growth. For example, Kanazawa (1982b) found that the addition of some vitamin C enhanced prawn growth while tan excess inhibited growth. Since prawns are slow feeders, most formulations are supplemented with vitamins at higher levels to

overcome the loss due to leaching. Crustaceans can absorb most minerals (except phosphorus) from seawater and consequently their dietary mineral requirements have not been well studied. However, it is not known whether crustaceans can absorb minerals at a sufficient rate to meet their requirements. Davis and Gatlin (1996) recommended that seven minerals should be included in crustacean feeds: calcium, copper, phosphorus, potassium, magnesium, selenium and zinc. Other minerals were found to have negative effects. Yet, in feed formulations percentage levels of calcium, phosphorus, magnesium, sodium and potassium are maintained along with other trace elements requirement. The quality and quantity of the ingredients influence the meeting of nutritional requirements in prawns. Some ingredients are very good such as soybean meal, which is rich in most amino acids. This can replace fishmeal in feed formulations up to 50% level and the cost of feed can be brought down. Similarly, the use of soy lecithin, which is rich in phosphatidyl choline/inositol, significantly promotes growth and survival.

The development and use of compound feeds have been major factors in the expansion of the industry and will continue gaining importance. Growing demand for formulated feeds will increase competition for component resources, particularly fish meal. There is an enormous but still unrealised potential to reduce production costs and improve the nutritional performance of compound feeds for prawn. Extensive research must continue to improve our knowledge of the nutritional requirements of prawn and to develop new diets that are species, area and even season specific. These diets could include innovative processing methods, lower-cost and innovative ingredients from various agriculture crops and by products from various meat processing industries, health additives and growth promoters that improve survival, growth, yield, food conversion ratio and disease resistance while reducing environmental concerns. Reducing the cost of feeds is an aspect critical to expanding the industry and improving its competitiveness relative to other protein sources, such as beef, pork and poultry. Low-pollution or 'environmentally friendly' feeds are being developed to reduce the environmental impacts of prawn feeds. Currently, emphasis is on cheaper feed formulations to minimise feed cost while optimising aquaculture production. In the future, emphasis will also be on

“least polluting” feed formulations to minimise environmental impacts with the greatest compatible aquaculture production.

2.2.1 Larvae feeding

Penaeid larvae hatch as a non-feeding stage called nauplius (ranges from 5-6 stages) and pass through three protozoal (PZ) stages and three mysis (M) stages before reaching the postlarval stage (PL). Penaeid hatcheries conventionally rear penaeid prawn larvae on microalgae (diatoms, flagellates, etc.) during the zoeal stage, and zooplankton (artemia, rotifers) during later stages (Hudinaga 1942; Aquacop 1983; Liao 1983; New et al. 2009). Penaeid prawn larvae obtain their food by filtering microalgae from the water at protozoal stages, and capturing zooplankton at mysis and post-larval stages. During planktonic stages, decapod crustacean larvae are chance encounter feeders and need a high density of food particles in suspension at all times. The cell size of microalgae used to feed early prawn larvae is generally between 5-20 µm in diameter, whereas the size of animal prey ranges from 70 to 500 µm. Artemia nauplii are the only realistic live prey for penaeid larvae until their early post-larval stages. Despite advances in the understanding of nutrition of adult and juvenile decapod crustaceans (Kanazawa 1984; Liao and Liu 1990; Guillame 1990; Chen 1990; Nelson et al. 2006), only limited information on specific dietary requirements of crustacean larvae is currently available. From the nutritional value of live algae successfully used in penaeid culture, it may be concluded that penaeid larvae require a protein level of between 23-55% of dry weight of diets (Liao and Liu 1990; Rodriguez et al. 1994). It is generally accepted that penaeid larvae and post-larvae have higher dietary protein requirement than juveniles and adults (Kanazawa 1984, 1990). However, Le Vay et al. (1993) demonstrated that *P. japonicus* larvae can be successfully reared to metamorphosis on the alga, *Chaetoceros gracilis*, which contains only 7% protein (dry weight). Jones et al. (1979) and D’Souza and Loneragan (1999) showed the importance of HUFA particularly EPA (20:5n-3) and DHA (22:6n-3) in *P. japonicas*, *P. semisulcatus* and *P. monodon* larvae using nutritionally defined microcapsules or microalgae with distinctly different fatty acid profiles. Some of the best algae used

today as food for prawn larvae are the green flagellate *Tetraselmis sp.* and the diatoms *Chaetocerus sp.* and *Thalassiosira weissflogii*. The use of mixed algal diets for penaeid prawn larvae often gives superior survival, growth and development compared to single algal species (Kuban et al. 1985) due to their more balanced nutrient content. The digestive system of penaeid prawn larvae is very simple and lacks a gastric mill and filter apparatus during herbivorous stages. The existence of the anterior midgut diverticulae (AMD) along with the small hepatopancreas (HP) was described by Lovett & Felder (1989, 1990) for *P. setiferus* and by Abubakr (1991) for *P. japonicus*, *P. monodon*, *P. kerathurus* and *P. vannamei*. Digestion is carried out by enzymes, released mainly from the AMD rather than the HP during early larval development (Abubakr 1991). At mysis stages, the teeth of the gastric mill are fully developed and thus, the larvae become increasingly carnivorous, retaining food longer and assimilating a higher percentage of energy from their prey (Jones et al. 1993). Although penaeid larvae exhibit low assimilation efficiency during herbivorous stages (Kurmalı et al. 1989), their survival on microalgae and artificial diets is thought to be due to their short gastroevacuation time (GET) and sufficient amount of digestive enzymes produced by the AMD (Abubakr 1991; Jones et al. 1993).

2.2.2 Post-larval feeding

Penaeids larvae after having gone through several planktonic stages, metamorphose into postlarvae and migrate to inshore brackish nursery grounds in estuaries. During this process, the prawn changes its habitat from planktonic to benthonic accompanied by changes in feeding habits and in the development of the digestive system (Pérez-Farfante 1969; Felder et al. 1985; Abrunhosa et al. 2006; Queiroz et al. 2011). These changes appear to take place during early post-larval development and not abruptly at metamorphosis to PL1 (Wickins 1976; Lovett and Felder 1989). Lovett and Felder (1990) assumed that ontogenetic changes occur in early post-larval development to adapt to a new feeding habit in order to maximize digestion and assimilation of food. Moreover, a “critical period” in early development of postlarvae (PL1±PL14 days old) has been identified with high mortality

(Wickins 1976; Bages and Sloane 1981) characterized by changes in the gut associated with digestive enzyme production levels and to the quantity or quality of some components of the diet (Le Vay et al. 1993; Rodriguez et al. 1994; Le Moullac et al. 1996; Lemos and Rodriguez 1998). Different artificial diets have been widely used in post-larval culture for early stages of development. However, little is known about whether post-larval enzyme response can be adapted to feed, and hence be reflected in growth, or whether physiological response is mainly controlled by ontogenetic development.

As regards protein requirement in the diet of *P.indicus*, it decreased as the age of the post-larvae progressed (Bhasker and Ahamed Ali 1984). Such lower protein requirements were reported for the juvenile also of other penaeid species: by Shewbart et al (1973) in *P. aztecus* (between 22 and 30%), Andrews et al (1972) in *P. setiferus* (30%), Sick and Andrews (1973) in *P. duorarum* (between 28 and 30%) and Sedgewick (1979) in *P. Merguiensis* (34 to 42%). The levels of carbohydrate and lipid present in the diet may also influence the dietary protein requirement. If the diet contains adequate levels of energy components the growth may be higher even at lower protein levels. It has been documented by several authors that feeding post-larvae of *Penaeus monodon* with highly unsaturated fatty acids (HUFA) enriched Artemia improved their quality, expressed as their ability to survive an exposure to a salinity shock (Tackaert et al. 1992; Rees et al. 1994; Kontara et al. 1995). Other lipid components such as the lecithin phosphatidyl choline (PC) may result in similar effects, as demonstrated by Camara et al. (1997) and Kontara et al. (1997, 1998), respectively, and Coutteau et al. (2000) for *P. japonicus* and *L. vannamei* post-larvae fed an artificial diet only varying in the level of soybean PC. For both species, 1.5% inclusion of PC in the diet resulted in a significant increase in stress resistance, expressed as mortality after exposure for increased time intervals to de-ionised water. Culture tests with vitamin C boosted microbound diets for post-larva of *P. monodon* (Merchie et al. 1998) and *L. vannamei* (Kontara 1997) confirmed the hypothesis that dietary ascorbic acid (AA) improved the overall physiological condition in post-larvae.

3 Systematics, biology and management of *Penaeus kerathurus*

3.1 Morphological aspects

The caramote prawn, *Penaeus kerathurus* (Forskål, 1775) belongs to the family Penaeidae (Fig. 3.1). Common names used locally to refer to this prawn are: caramote (France), langostino (Spain), mazzancolla (Italy), caramote (England), gombri and shrimp (Tunisia).

It is a large prawn exceeding 22 cm in total length (from the tip of the rostrum to the end of the telson), it has a beige coloured esoskeleton with dark transverse bands forming separate spots on the side of the body and with blue uropodes. The first three pairs of legs have pincers. The telson is sharp and has three sides of movable spines. Laterally, the first abdominal segment covers the second. The rostrum is short and barely goes beyond the eyes; it has eight to thirteen dorsal spines and just one ventral spine. The caudal end is sky blue, bordered with red. The appendages are yellowish. Behind the last dorsal tooth, there is a double hull with a typically deep median furrow extending to the posterior edge of the carapace; a long ridge runs parallel to the rostrum. In the hepatic region there is a ridge in the anterior – ventral direction; the gastrointestinal front peak is present. The abdomen has a dorsal keel in all segments and ends with a spine on the sixth segment. The body of the caramote is segmented and protected by a chitinous carapace, and can be divided into two distinct regions: the carapace, composed of 14 segments, protected on the back and sides by a vast carapace partly merged with the ventral segments and ending before a rostrum; the abdomen, a hinged piece that consists of six mobile segments and an end piece (telson) where the anus opens. The sixth segment has two fan-like appendages on each side (uropods), which together with the telson form the swimming apparatus. Sexes can be identified by the presence of secondary sexual characters. In male, the endopods of the first pair of pleopods (swimming legs) appear initially as leaf-like and later develops into semi-cylindrical tube. The tube-like structures fuse together when the prawn attains maturity and it is called as "Petasma". In female, the external genitalia is called as "Thelycum", which is situated between the

bases of last three walking legs. The thelycum is only a pouch, where the spermatophores are stored at the time of mating.

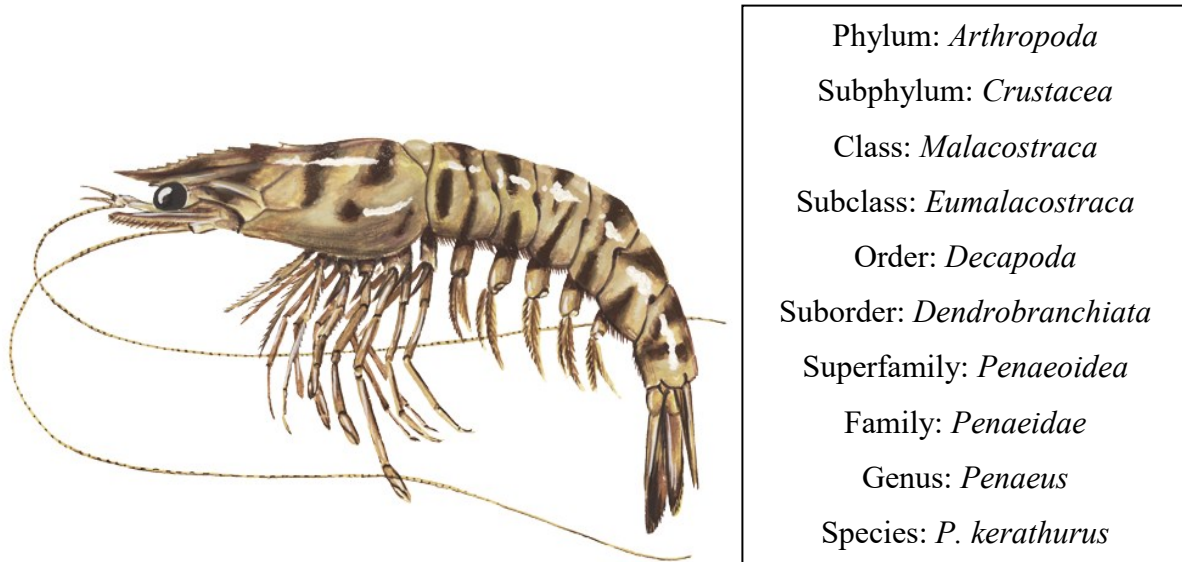


Fig. 3.1 *Penaeus kerathurus* (Forskål, 1775) and its taxonomic group

3.2 Ecology

The caramote prawn is a benthic crustacean species that prefers sandy and muddy low depth (0-90 m) bottom (FAO 2007) adapted to life from brackish estuaries to water with high salinity. It is found throughout the Mediterranean Sea (Fig 3.2), including the Marmara Sea, and in the Atlantic Ocean from Portugal to Angola (D'Udekem d'Acoz 1999). The best catches are recorded at shallow depths reflecting the coastal nature of this species (Azouz 1972; Ben Mustapha 1967). Very small juveniles lead a sedentary life near the shores at very shallow depths. At 5 to 8 cm juveniles begin migrating to a depth of about 50 meters where they await the spring season to reach the spawning grounds. Indeed, in relation to bathymetric distribution during the horizontal migration Ben Meriem (1998) reported that the young fraction of the population stays strictly within the coastal area while the largest prawns

occupy the deepest areas. During the day it reacts to the solar irradiance with a negative phototropism (vertical migration) and goes towards the bottom, sinking completely into the sand or mud, hiding among the vegetation such as algae, seagrass or plant debris. On the contrary, at night the prawn temporarily leaves this area to swim and make incursions into neighbouring areas to feed (Ben Mustapha 1967).

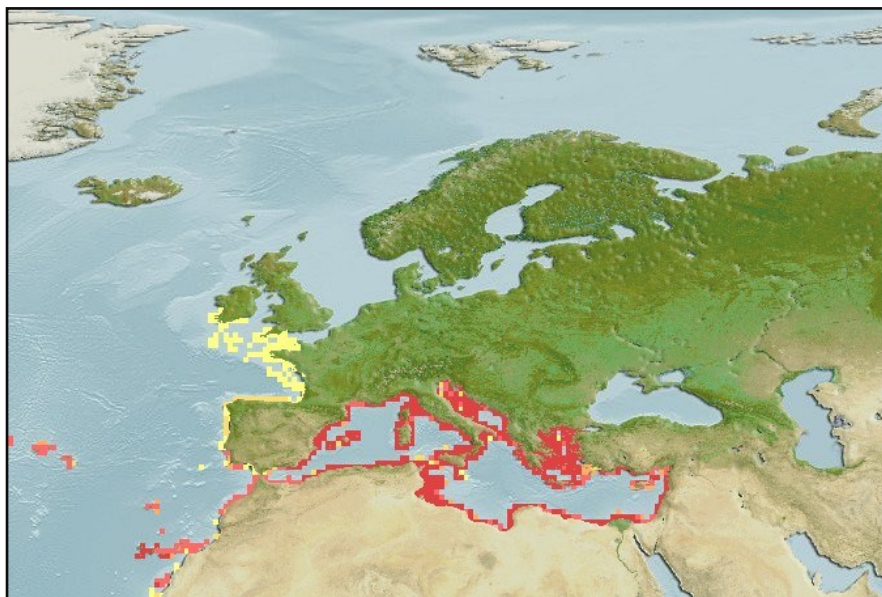


Fig. 3.2 Map of caramote prawn distribution (Jaziri et al. 2011).

3.3 Biology

The size at first sexual maturity of *P. kerathurus* female is about 18 cm total length (Turkmen et al. 2007; Conides et al. 2008). Nurseries of *P. kerathurus* are in very shallow water near the shore and juveniles migrate later to 25m pits at first sexual maturity size. The first wave of recruitment to the commercial stock occurs in August (Vitale et al. 2010).

A penaid female can produce between 68,000 and 1,900,000 eggs per spawning (Uthirvel 2003). The degree of ovarian maturation in a caramote prawn female is divided in four stages of development (Fig. 3.3): Stage I (immature/undeveloped) – ovaries thin, translucent and hardly visible; Stage II (early maturing/developing) – ovaries yellow, increasing in size and visible through the exoskeleton

with the anterior and middle lobes starting to develop; Stage III (late maturing/nearly ripe) – ovaries yellow, middle and anterior lobes fully developed; Stage IV (mature/ripe) ovaries dark yellow with the prawn ready to spawn.

Life cycle (Fig. 3.4) is characterized by a larval stage that takes place away from the coasts, and a post larval and a juvenile stage that take place near the coast or in estuaries with low salinity and high productivity (Arculeo et al. 2011; Cannizzaro et al. 2011). After spawning, it takes about 12 to 15 hr before *P. kerathurus* eggs hatch to the nauplius stage; there are six naupliar substages (N1, N2, N3, N4, N5, N6), a nauplius moults 5 times to N6 within 48 to 56 hr. Nauplii at substage N6 moult to the protozoa stage, composed of three substages (PZ1, PZ2, and PZ3), all of which are completed within 5 to 6 days. The next and last larval stage is the mysis, which it also has 3 substages (M1, M2, and M3), which are completed in 4 to 5 days. Finally, the post-larval stage follows.

The naupliar larvae do not feed as they possess yolk, on which they thrive. When they metamorphose into protozoa stage, they become filter feeders. The mouth part has a series of setae, which serve as a screen in filtering microscopic algae present in sea water. By their active swimming, larvae move around and actively feed on phytoplankton (Liao 1985). Larvae become carnivorous feeding on zooplankton in a successive stage, the mysis stage (Lavens et al. 2000), while post-larvae and adults are omnivores feeding on algae, crustaceans, molluscs and annelids (Ismael and New 2000; Karani et al. 2005, Prato et al. 2011).

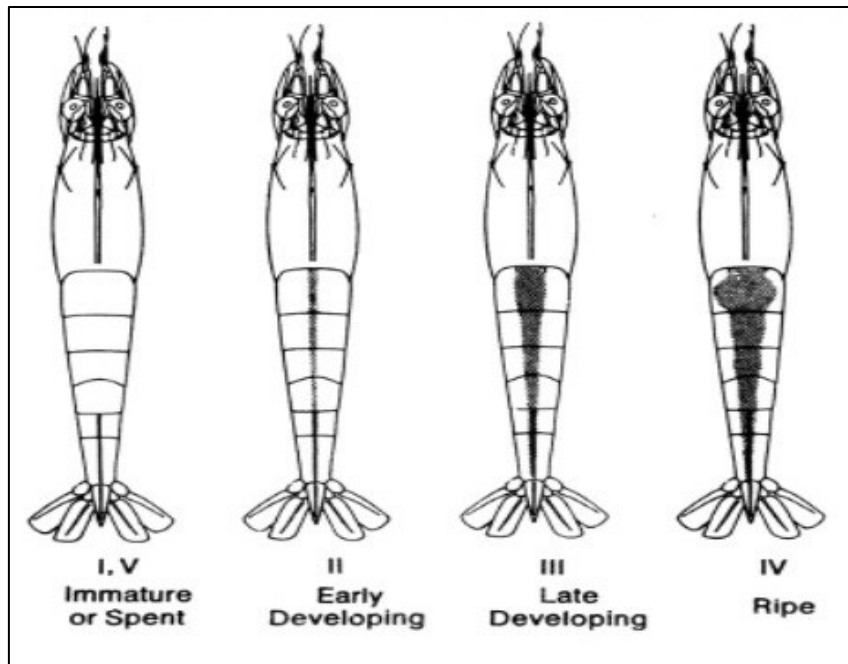


Fig. 3.3 Degree of ovarian maturation in a prawn female.

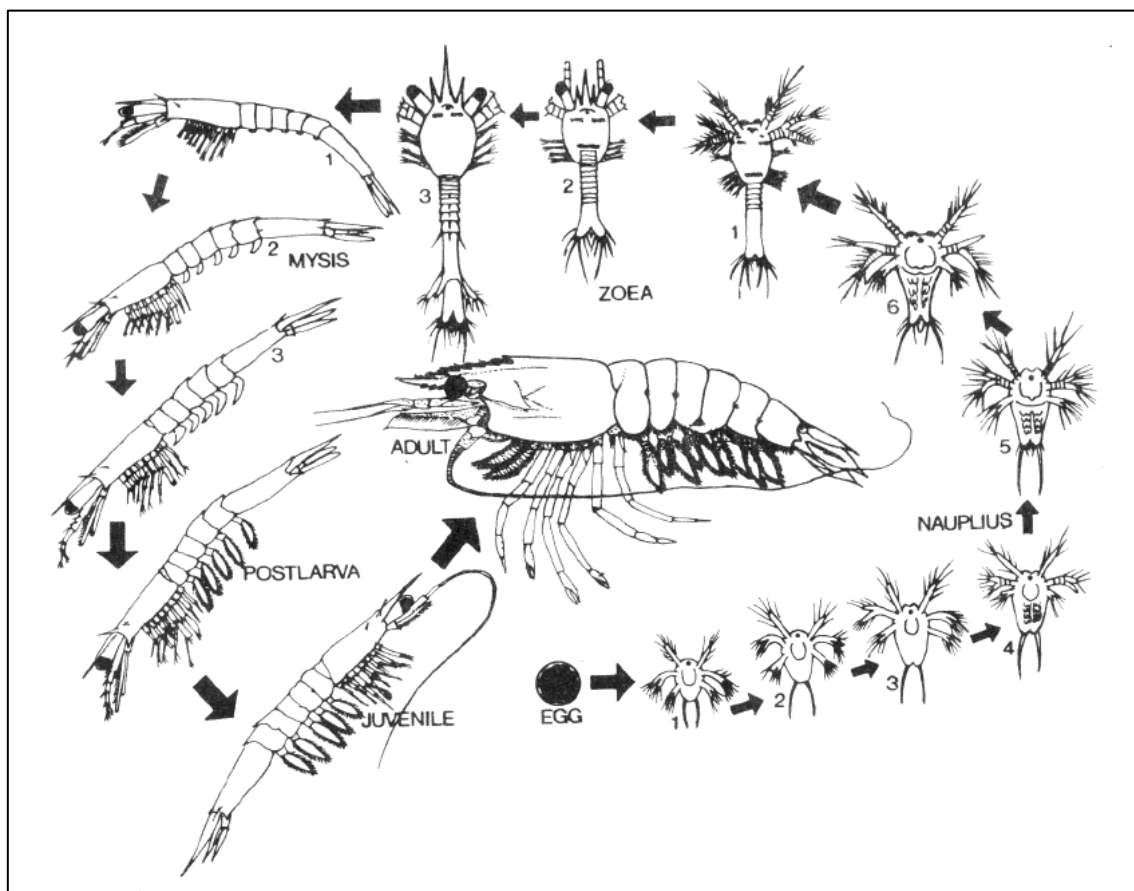


Fig. 3.4 The life cycle of the caridean prawn.

3.4 Stock evaluation and exploitation

Fisheries in Sicily (Italy) and Libya (west coast) of this species are mainly performed by gill and trammel nets (Vitale et al. 2010). Monitoring of catch and fishing effort of *P. kerathurus* off the west coast of Sicily (Italy) between Cape San Marco and Cape Granitola from 1998 to 2010 shows that the catch per unit effort decreased substantially from 2002 to 2009 with the exception of 2005, probably due to favourable climatic conditions (Fig. 3.5) (Cannizzaro et al. 2011). The exploited stock off the south-western Sicilian coast is composed of two dominant modes (i.e. one and two years old individuals) plus a few individuals up to three years old (Vitale et al. 2010). In the Gulf of Gabès carapace prawns are captured by trawlers and artisanal fisheries, with most of the catch by trawlers. Fishing mortality (F) values differ significantly depending on size. F is very low (< 0.1) for very young individuals with a size of less than 100 mm, but it increases rapidly at the size of first sexual maturity (127mm) when it reaches a value of around 0.4. The F values of trawlers on the classes below 120 mm remain relatively low. In contrast, fishing mortality by artisanal coastal fisheries, although lower than that caused by trawlers, shows the capture of a larger percentage of juveniles (= immature) compared to trawlers.

The low degree of genetic variation found in this species and the moderate genetic heterogeneity impose extreme caution in the use of this important resource for fishing purposes (Arculeo et al. 2011).

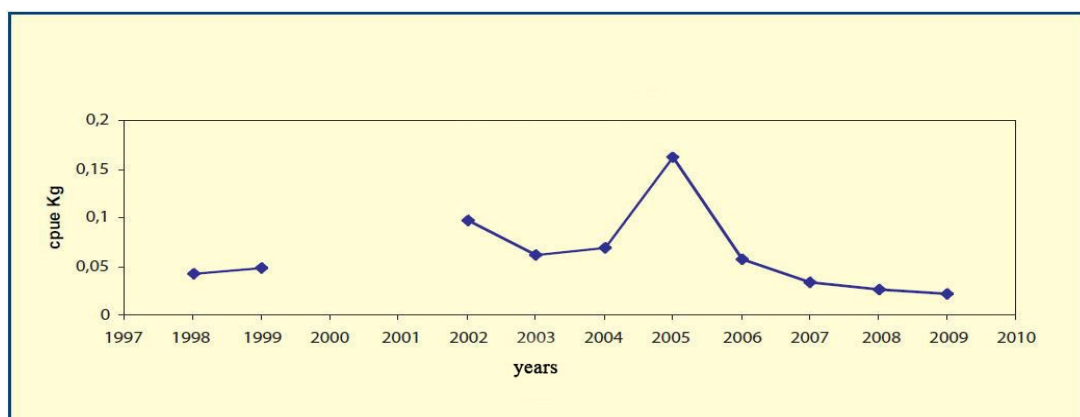


Fig. 3.5 CPUE in weight (kg) of *P. kerathurus* (Cannizzaro et al., 2011)

4 Captive breeding of the caramote prawn (*Penaeus kerathurus*) for restocking purpose: effect of single and mixed-algae diets on larval growth and survival

Abstract

Attempts to breed crustacean species of commercial interest in order to promote the conservation, restoration and enhancement of the stocks are increasing. Optimization of diet for larval growth is a crucial step for successful actions. Here we focus on the caramote prawn (*Penaeus kerathurus*), a primary target of small-scale fishery because of its high commercial value in the North Mediterranean region and particularly in Italy. Three species of microalgae (*Isochrysis galbana*, *Nannochloropsis oculata*, and *Chaetoceros calcitrans*) with different fatty acid contents were used to feed caramote prawn larvae as monospecific diets. Further, *C. calcitrans* in combination either with *I. galbana* or *N. oculata*, were tested as mixed diets. Measurements of developmental performance were taken until the larvae metamorphosised to Mysis 1, while survival rate was estimated at day 10 of benthonic post-larval stage. Larvae fed on mixed diets performed as well or slightly better than those fed on single *C. calcitrans* and *N. oculata* diets both in terms of development and survival, while larvae fed with *I. galbana* diet performed worse overall. Diets that performed best (*C. calcitrans* and *I. galbana* mix) are known to have a higher proportion of the essential fatty acids eicosapentaeneic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), respectively. We suggest that mixed-algae diets could improve the performance of larvae by providing a wider availability of fatty acids.

Keywords larval development, restocking, essential fatty acids, phytoplankton, diet, aquaculture, crustaceans

4.1 Introduction

The caramote prawn *Penaeus kerathurus* is an Atlantic-Mediterranean benthic crustacean species that prefers sandy and muddy low depth (0-90 m) bottoms (FAO 2007). The life cycle is characterized by a larval stage that takes place away from the coasts, and a post-larval and juvenile stage that takes place near the coast or in estuaries with low salinity and high productivity (Arculeo et al. 2011; Cannizzaro et al. 2011b). There are six naupliar substages, after that nauplii moult to the protozoa stage, composed of three substages (PZ1, PZ2, and PZ3). The last larval stage is the mysis, which also has 3 substages (M1, M2, and M3). Finally, the post-larval stage follows. When larvae metamorphosize into protozoa stage (naupliar larvae do not feed as they possess yolk) they become filter feeders and feed actively on phytoplankton (Liao 1985).

P. kerathurus is one of the primary targets of the small-scale fishery that operates with gillnets because of its high commercial value in some Mediterranean coastal regions and particularly in Italy. Intense exploitation rates since the 1970s resulted in a significant decrease in *P. kerathurus* stock abundance, reflected in declining annual landings in Spain, Italy and Greece, (Rodríguez 1977; Scordella and Lumare 2001; Conides et al. 2006).

Nowadays, depletion of aquatic ecosystems is a major global challenge. Recently SDG 14 (Sustainable Development Goals) of United Nations “Agenda 2030” aimed at sustainably managing and protecting marine and coastal ecosystems to avoid significant adverse impacts by strengthening their resilience, and taking action for their restoration. Such objectives, in turn have boosted science-based management plans in order to restore fish stocks in the shortest feasible time, at least to levels that can produce maximum sustainable yield as determined by their biological characteristics. The juvenile or adult aquaculture of this species is not economically convenient for commercial purposes because of the slow growth rates and the limitations and costs associated with the management of the hatchery (Lumare 2001). However, aquaculture could still be useful when these species are bred in targeted restocking programs to be carried out in selected areas, in order to strengthen the wild stocks and thereby increase catches (Cannizzaro et al. 2011a, b; Vitale et al. 2013), as in the case of other

decapod crustaceans such as the European lobster *Homarus gammarus*, (e.g. Bannister et al. 1994; Agnalt et al. 2004; Schmalenbach et al. 2010; Carere et al. 2015; Aspaas et al. 2016; Agnalt et al. 2016). According to Herrnkind and Butler (1994) or Phillips *et al.* (2003), less than 1-3,5% larvae of decapods reach the adult stage. Mortality rates are not constant over the complete crustaceans' developmental period but decrease with time. The management strategies based on active restocking aim to reduce the high natural mortality of crustaceans first life stages. As stated by McConaughy (1992), indeed larval recruitment success can be measured by the number of larvae surviving the planktonic period that will subsequently settle in the adult habitat.

A major challenge in prawn aquaculture is the determination of the optimal diet and food regime for larvae. Such information on several target species, including *P. kerathurus*, is still scanty. Phytoplankton is the main food of larval stages for most of marine crustaceans (Preston et al. 1992; Narciso and Morais 2001) and differences in the composition of protein, lipid and in particular fatty acids in the algal diet were associated with different survival and growth rates (D'Souza and Loneragan 1999). Mixed diets resulted in higher survival and faster development of *P. monodon* larvae compared to monospecific diets (Kurmaly et al. 1989). In a controlled environment a balanced diet that supports a fast growth and a high survival is therefore essential.

The aim of this study was to assess the performance in terms of growth and survival of *P. kerathurus* larvae fed with monospecific diets and different combinations of three different species of algae (*Isochrysis galbana*, *Nannochloropsis oculata*, *Chaetoceros calcitrans*) commonly used to feed prawns during early stages (D' Souza and Loneragan 1999, Brown 2002, Piña et al. 2006). These algae are characterised by high but different contents of fatty acids, which appear to be involved in larval prawn survival and development (Jones et al 1979, 1997; Brown et al. 1989; Piña et al. 2006).

4.2 Methods

4.2.1 Algal culture

Cultures of the microalgae *I. galbana*, *N. oculata*, and *C. calcitrans* obtained from the Italian National Institute of Health, Department of Health and Environment, Rome, were grown in laboratory in one litre conical flasks (Fig. 4.1) as starters for three 50 l plexiglass tube (Fig. 4.2) filled with natural seawater filtered through 1µm cartridge and enriched with f/2 medium. The cultures were aerated and maintained at 20 °C. Cellular growth and protein production occur during the plants dark cycle. Moreover, many species of phytoplankton hardly tolerate 24-hour photoperiods (Guillard RRL, 1975). According to that, illumination was provided by two T8 5000 lm fluorescent lamps (Osram Daylight) set on the side of each tube, ensuring a 12:12 light-dark cycle. Cell density in the cultures was monitored with a Neubauer haemocytometer.



Fig. 4.1 Conical flasks for phytoplankton laboratory culture (top).



Fig. 4.2 Phytoplankton 50 litres plexiglass tubes with f/2 medium (right).

4.2.2 Larval culture

Larvae were obtained from wild females collected by fishermen from the coast of northern Lazio, Italy (42,0°N 11,8°E – 42,3°N 11.6°E). *P. kerathurus* stage IV (mature) and III (late developing)

females bearing spermatophores were selected according to Primavera (1985), using the *in vivo* classification by looking at the ovaries externally through the dorsal exoskeleton, and were transferred to 90 dm³ floating tanks with a gentle lateral flow immersed in seawater with a salinity of 35‰. A screen, nylon plankton 125-µm mesh, was placed over the outlet hose to prevent eggs from being pumped into the filter. Since spawning females could carry infective agents, which could affect eggs and subsequent larval stages, they were left in a disinfecting solution of hydrogen peroxide 30% (20ml L⁻¹) for 20 min, with aeration provided during disinfection. Normally, in a captive environment, shrimps do not spawn, but resorb their eggs in a few days (Cook 1967). In order to prevent such a risk, spawning was induced through the unilateral eyestalk ablation and a progressive thermal shock, from 22°C to 26°C in 12 hours (Lumare 1979; Narciso and Morais 2001). Females were kept in the shadow during the whole incubation period (48 hours) using dark panels placed over the spawning tanks. Spawning took place during the night and the next morning the eggs were collected with a 125-µm mesh and were transferred in a 2 liters conoid hatchery (Fig. 4.3) with a bland bottom aeration. After the eggs hatched, nauplii were siphoned and concentrated at a density of 250 larvae L⁻¹ in nine 15 liters tritan carboys (Fig. 4.4) filled with 35‰ salinity filtered seawater at 25°C. Aeration was supplied by an air stone in the neck of the carboy providing mixing as well as oxygen. This procedure resulted in good water circulation which, coupled with the sloping shoulders of the carboy, prevented the food from settling out. Each day one third of water was replaced and a screen was placed over the mouth of the siphon to prevent the loss of larvae. The larvae were fed to excess with selected algal species at densities of 1x10⁵ cells ml⁻¹ (Platon 1978; Villegas and Kanazawa 1979) and this concentration was maintained daily at protozoa stage. Larvae from each carboy were collected twice a day (in the morning and evening) with a 100 ml beker and their guts and faeces were examined with optical microscope to confirm that the different alga cells were being ingested and digested (Fig. 4.5). When the shrimp reached mysis I, freshly hatched *Artemia* nauplii were added daily at a density of 5-10 individuals ml⁻¹ (Platon 1978; Narciso and Morais 2001).



Fig. 4.3 2 litres conoid eggs hatchery (left).

Fig. 4.4 15 litres tritan carboys for larvae breeding (top).



Fig. 4.5 PZ1 feeding on microalgae (evidence of larval excrement)

4.2.3 Experimental procedure

Five experimental trials coinciding with five different spawnings (larvae used in each trial were each from a single different female) were carried out in July and August. In the first three trials (1, 2, 3) larvae were fed with the single-algal diet *C. calcitrans*, *I. galbana* and with the mixed-algae diet (a combination of both microalgae). In the fourth trial (4) the larvae were fed the single-alga diet *C. calcitrans*, *I. galbana* and *N. oculata*, and no mixed-algae diet were used. In the last trial (5) we only used algae that performed well in the previous trials (see results), therefore larvae were fed the single-alga diet *C. calcitrans*, *N. oculata* and with the mixed-algae diet (a combination of both microalgae). There were two larval replicates per treatment, therefore in each trial a total of 6 different carboys were used, two for each diet treatment. Overall, 30 carboys were used.

4.2.4 Data collection

Observations were made until the 10th day of post-larval stage twice a day (in the morning and evening) taking three 300 ml subsamples from the different carboys and counting larvae under a stereomicroscope. A development index (DI) was calculated by the following formula (Villegas and Kanazawa 1979): $DI = A / \text{total number of larvae staged}$, where $A = \text{stage value} \times \text{number of larvae at that stage}$. The stage values increase as the larvae moult through the protozoal substages and metamorphosize to M1 with values for PZ1 = 1, PZ2 = 2, PZ3 = 3 and M1 = 4. Therefore, the greatest the proportion of later-stage larvae in a treatment, the highest the DI. Survival rate (SR) was estimated by the total number of hatched nauplii and the number of 10 days benthonic post-larvae.

4.2.5 Data analysis

At the beginning the effect of female and carboy on DI and SR was checked. Then an ANOVA model for repeated measures with diet as between-subjects factor (five levels, i.e. the five trials) and developmental stage as within-subjects factor (four levels, i.e. the four developmental stages PZ1, PZ2, PZ3, M1) was run to test for the effect of diet on the DI and the possible interaction between the two main factors. The effect of diet on SR could only be tested on those three females that had surviving offspring (trials 1, 4, and 5). To test this effect, a simple ANOVA model followed by Fisher's LSD post hoc tests was run. Moreover, DI and SR were observed separately for each trial by means of ANOVAs. The analyses were performed with Statview II (Abacus Concepts, CA, US) after log (for DI) and angular (for SR) transformation.

4.3 Results

The effect of female and carboy was not significant for both factors for DI (repeated measures ANOVA $F_{4, 69} = 1.5$; $p = 0.25$; $F_{5, 66} = 2.6$; $p = 0.06$, respectively). However, female, but not carboy effect ($F_{5, 22} = 0.37$; $p = 0.86$), was highly significant for SR ($F_{4, 23} = 43.8$; $p < 0.0001$), even when removing two females that had no offspring survival ($F_{2, 15} = 31.1$; $p < 0.0001$) due to a system failure. There was a significant effect of diet both overall ($F_{4, 69} = 6.2$; $p = 0.016$), Fig. 4.6, and in interaction with developmental stage ($F_{12, 69} = 4.3$; $p < 0.001$, Fig 4.6): starting from PZ2, the larvae fed on *I. galbana* did always worse than any other diet, while in PZ1 they all performed similarly. The mixed-algae diets induced a faster development than *N. oculata* and *C. calcitrans* single-alga diets, but the results did not reach statistical significance ($p > 0.05$, except for Chaeto vs Iso $p = 0.05$).

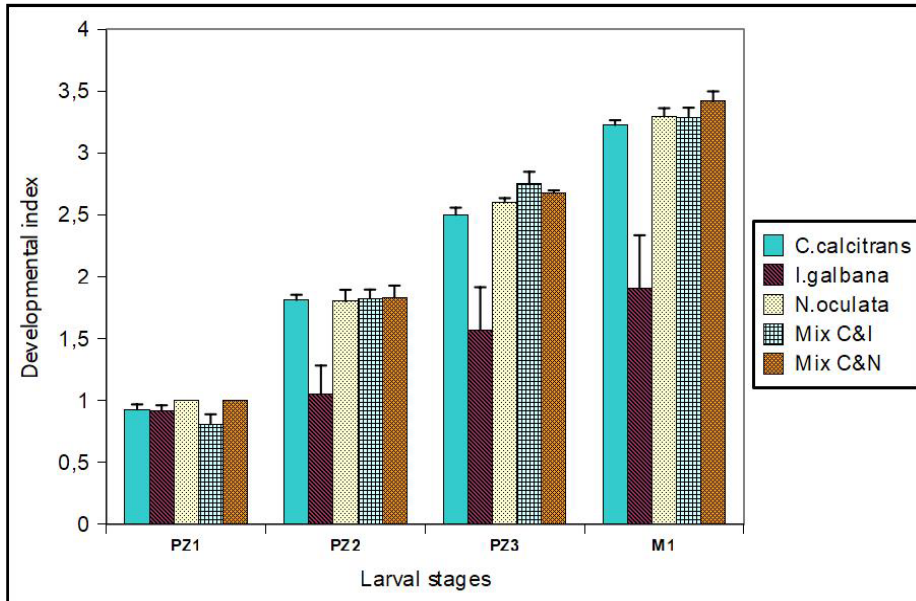


Fig. 4.6 Developmental index across different larval stages of *P. kerathurus* in relation to diet (Mix C&I= mix *C. calcitrans* & *I. galbana*; Mix C&N= mix *C. calcitrans* & *N. oculata*). Values are means $\pm$ SEM. Total number of carboys = 30. See method section for details.

Analysing the single trials separately, significant differences in DI between algal diets were detected from the second moult (PZ2) in Trial 1, Trial 2, Trial 4 and Trial 5, while a significant interaction effect between diet and developmental stage was only present in Trial 4 (Trial 1, diet effect: $F_{2, 9} = 80.0$; $p = 0.002$; Trial 2, diet effect: $F_{2, 9} = 71.7$; $p = 0.003$; Trial 3, diet effect: $F_{2, 6} = 13.5$; $p = 0.07$; Trial 4, diet effect: $F_{2, 9} = 64072$; $p < 0.0001$; diet * developmental stage: $F_{6, 9} = 101.2$; $p < 0.0001$; Trial 5, diet effect: $F_{2, 9} = 75.9$; $p = 0.003$. Fig. 4.7.); larvae fed *I. galbana* in Trial 4 died at PZ2 stage (Fig. 4.7 and 4.9).

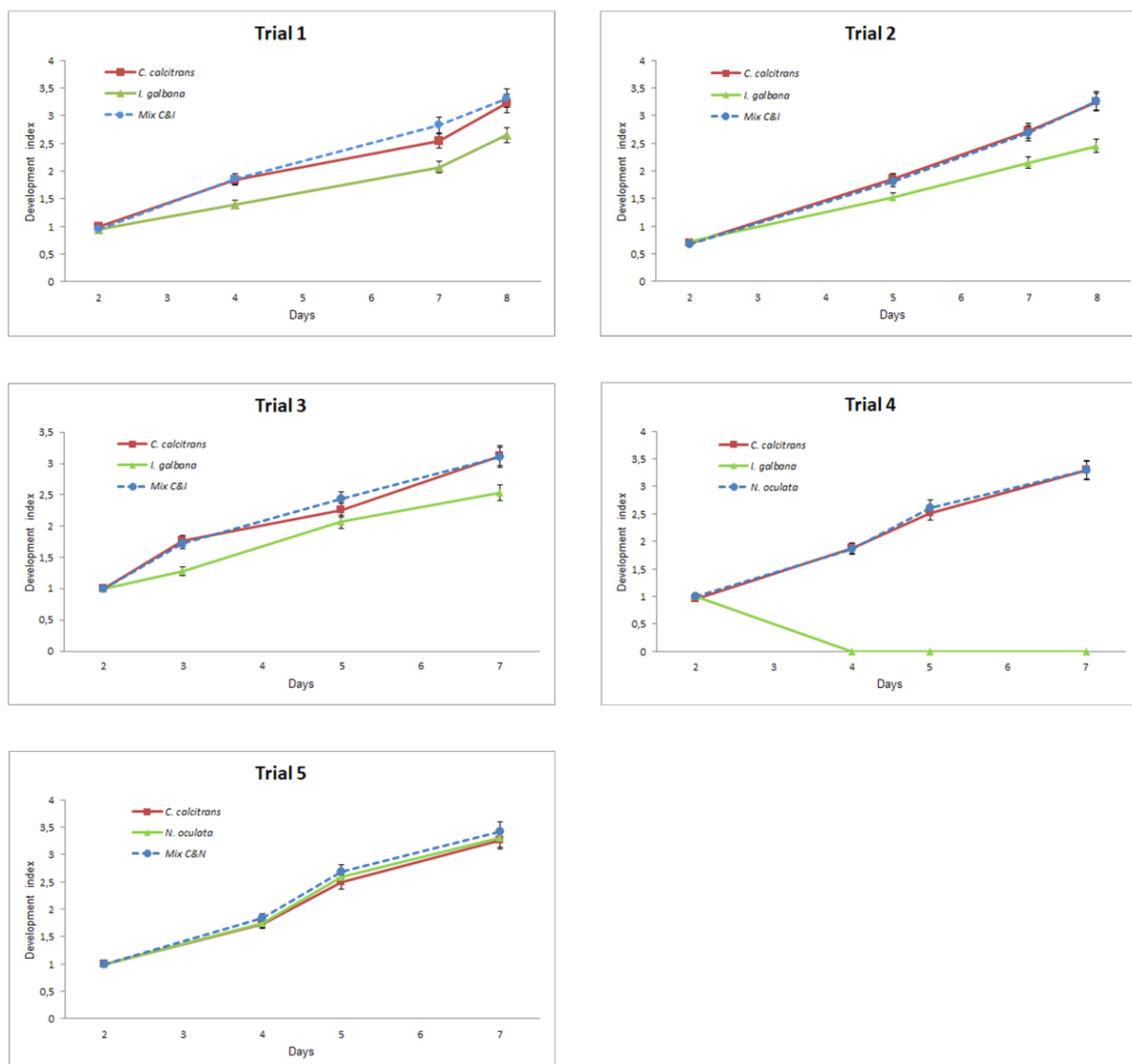


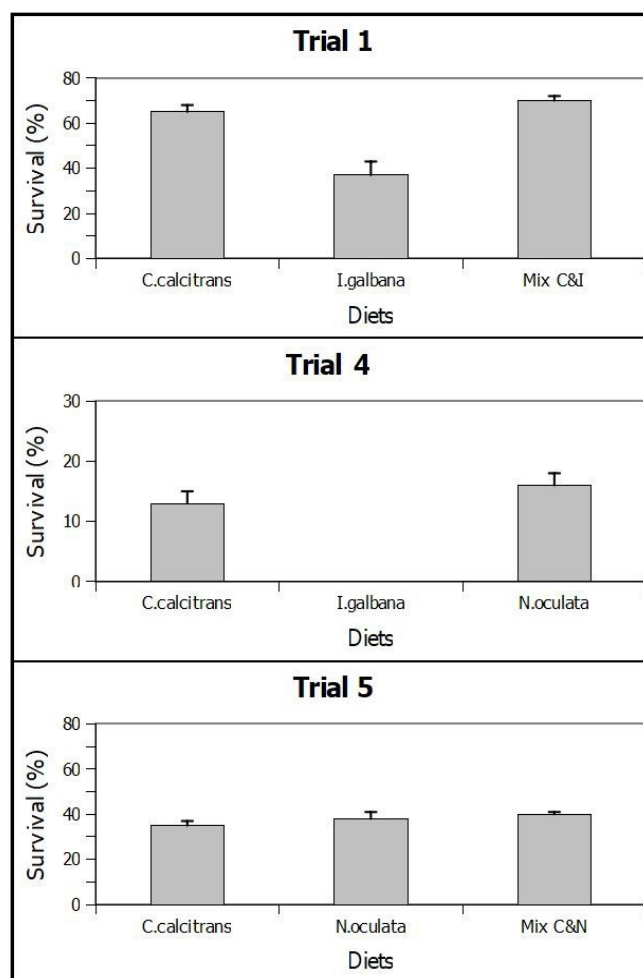
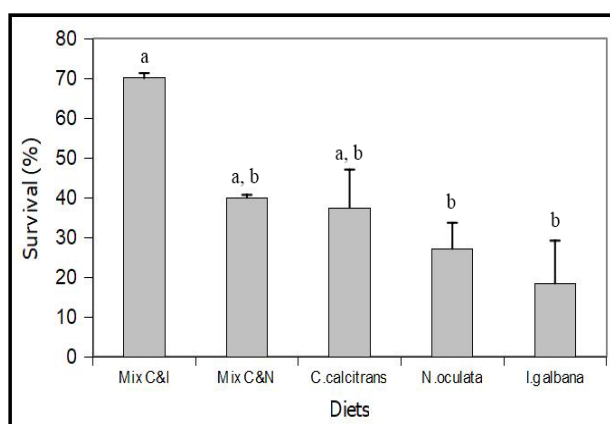
Fig. 4.7 Change in mean (± 1 SE) developmental index over time of *P. kerathurus* larvae in relation to diet in trials 1, 2, 3, 4 e 5 (Mix C&I = *C. calcitrans* & *I. galbana*; Mix C&N = *C. calcitrans* & *N. oculata*). Each point corresponds chronologically to the four larval stages of Fig. 1. Total number of carboys/trial = 6.

As regards the effect of diet on SR, unfortunately, due to a system failure, larvae died on day 10 in Trial 2 and Trial 3, losing data on SR. So we could only use trials 1, 4, and 5. The overall effect was close to significance ($F_{4, 13} = 2.7$; $p = 0.077$, Fig. 4.8) but two post hoc tests were statistically significant and another one was close to significance, each of them comparing *C. calcitrans* & *I. galbana* diet with the other single-alga diets (mix C&I vs *Isochrysis*, $p = 0.008$, mix C&I vs *Nannochloropsis*, $p = 0.02$ and mix C&I vs *C. calcitrans* $p = 0.055$, Fig. 4.8). Analysing the single

trials separately, SR within each trial was higher with the mixed-algae diets (C&I) and *N. oculata* or *C. calcitrans* single diet than with *I. galbana* diet (Trial 1, diet effect: $F_{2, 3} = 41.4$; $p = 0.006$; Fisher's PLSD: *C. calcitrans* vs *I. galbana* $p = 0.006$; *I. galbana* vs Mix C&I $p = 0.003$. Trial 4, diet effect $F_{2, 3} = 52.3$; $p = 0.005$; Fisher's PLSD: *C. calcitrans* vs *I. galbana* $p = 0.004$; *I. galbana* vs *N. oculata* $p = 0.002$. Trial 5, diet effect: $F_{2, 3} = 3.5$; $p = 0.16$. Fig. 4.9). In Trial 4 SR was the same for *C. calcitrans* and *N. oculata* diet, instead all larvae fed on *I. galbana* died. No significant differences in SR were detected in Trial 5 between larvae fed *N. oculata*, *C. calcitrans* and both mixed-diet (Fig. 4.9). Overall, the SR of larvae was lower in Trial 4 and Trial 5 than in Trial 1, with almost double proportion of larvae dying.

Fig. 4.8 Mean (± 1 SE) percent survival of *P. kerathurus* larvae in relation to diet (Mix C&I = *C. calcitrans* & *I. galbana*; Mix C&N = *C. calcitrans* & *N. oculata*). Values that do not share the same letter are significantly different from each other. Total number of carboys = 30.

Fig. 4.9 Mean (± 1 SE) percent survival of *P. kerathurus* larvae in trials 1, 4 e 5 in relation to diet (Mix C&I = *C. calcitrans* & *I. galbana*; Mix C&N = *C. calcitrans* & *N. oculata*). Total number of carboys/trial = 6.



4.4 Discussion

P. kerathurus larvae performed better on a mixed diet of *C. calcitrans* and *I. galbana* than on single-alga diets, while *I. galbana* alone always performed worse. Indeed, larval development was similar in the different diets apart from *I. galbana* diet, which performed worse than the others from PZ1 stage, but only a *Chaetoceros* & *Isochrysis* mix diet showed a significant effect improving survival. Indeed, the larvae fed a mixture of algal species performed better than those fed only one algal species, confirming previous observations (Becker 2004). Monospecific diets may cause nutritional deficiencies affecting larval survival and growth because of the lack or inadequate content of some essential nutrients (Piña et al. 2006). The gross lipid, protein and carbohydrate compositions of the three algal diets, in accordance with their percentage of dry weight that characterises *C. calcitrans*, *I. galbana* and *N. oculata* (Brown 1991), probably did not contribute to the observed differences in development and larval mortality. Indeed, previous studies have found that the gross composition of algae alone could not explain differences in the survival and growth of prawn and bivalve larvae (e.g. Tobias-Quinitio et al. 1982; Webb et al. 1983). Instead, we argue that differences in larval survival and development of *P. kerathurus* could be due to different contents of fatty acids in the algal diets we used. Penaeid larvae have an absolute requirement for long-chain unsaturated fatty acids, especially for C20 and C22: n-3 and n-6 (Jones et al 1979, 1997; Brown et al. 1989; Piña et al. 2006) and significant concentrations of EPA (20:5n-3) are present in *C. calcitrans* and *N. oculata*, whereas high concentration of DHA (22:6n-3) are found in *I. galbana* (Volkman et al. 1989). In this work *P. kerathurus* larvae fed on a diet with a large proportion of EPA (*C. calcitrans* and *N. Oculata* diets) appear to induce a good growth; moreover, the absence of DHA contents in the diet could explain the high mortality in diets with *I. galbana* alga. Indeed, the presence of DHA in lipids of *P. kerathurus* post-larvae, even when fed a diet lacking this particular fatty acid, indicates its essentiality for larvae of this species (Mourete et al. 1995).

Particle size is an important consideration in the rearing of larval forms of animals and some authors attributed the poor performance of prawn larvae to the large size of the algal cells in the diet (e.g.

Tobias-Quinitio et al. 1982; Sánchez 1986). *C. calcitrans*, *I. galbana* and *N. oculata* are very small and similar in size (2 to 6 µm), therefore it is unlikely that differences in performance between these three algal diets are due to the size of the algal cells. Indeed, according to Yufera et al. (1984) *P. kerathurus* larvae starting from PZ2 stage are able to ingest rotifers, which have a size larger than 100 µm.

The survival but not the development of prawn larvae varied greatly between spawning (trials), indicating a strong maternal effect. A similar variation in survival (but also development) of the larvae from different spawning fed the same diet has been recorded for *P. japonicus*, *P. semisulcatis* and *P. monodon* larvae (D'Souza 1999) and for *Metapenaeus ensis* larvae (Chu and Lui 1990). Most of this variation may be due to differences in quality of the nauplii from different spawnings related to the broodstock age, size, nutritional condition, season of spawning and time spent in maturation tanks (Crococ and Coman 1997; Arcos et al. 2003; Racotta et al. 2003).

Future experiments should examine the effect on *P. kerathurus* larvae of other microalgae rich in EPA and DHA, like *Thalassiosira tertiolecta* or *Tetraselmis suecica*, and continue with further experiments using *N. oculata*, *C. calcitrans* and *I. galbana*, as well as new combinations of mixed diets. More trials and a higher sample size are necessary in order to analyse the right role of these fatty acids into development and survival of *P. kerathurus* larvae as well as to confirm the general benefit of a mixed diet compared to a single-alga diet. Finally, D'Souza and Loneragan (1999) found that the arachidonic acid (ARA, 20:4n-6) appears to correlate with the growth of *P. semisulcatis*, *P. monodon* and *P. japonicus*, therefore it would be interesting to investigate also the role of this acid on *P. kerathurus* larvae.

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5 Restocking trial of the caramote prawn (*Penaeus kerathurus*) and a comparison of different diets on post-larval growth and survival

Abstract

A restocking trial of caramote prawn (*Penaeus kerathurus*) was carried out in 2015 along the coastal area of northern Lazio, Italy. Post-larvae were produced in a hatchery from wild breeders caught in the same area. A total of 460,200 eggs were released by 5 females, from which 118,922 nauplii hatched (29%). About 10,000 nauplii reached the PL20 sub-stage and they were sowed by scuba divers on a shallow sandy bottom. Simultaneously, a 60 PL20 post-larvae were kept in the hatchery for 8-weeks to test the effect of four different diets (frozen mussels; frozen mysis; artificial wet mash; dry pellet) on growth performance and survival, with the aim of optimizing rearing time and improving future restocking programs. Diet based on dry pellets showed the best results for *P. kerathurus* post-larvae gain in size and percentage of survival, while the worst performance was recorded with the diet based on mysis.

Keywords

Penaeus kerathurus, restocking, post-larval development, growth, decapod crustaceans, diet

5.1 Introduction

The carapace prawn *Penaeus kerathurus* is an Atlantic-Mediterranean benthic crustacean species that prefers sandy and muddy low depth (0-90 m) bottoms (FAO 2007). Coastal fishery and in particular marine crustaceans of commercial interest such as shrimps and prawns are largely affected by over-fishing, illegal fishing and pollution (Conides et al. 2011). This species is one of the primary targets of small-scale fishery, which operates with gillnets because of its high commercial value in some Mediterranean coastal regions and particularly in Italy. Intense exploitation rates since the 1970s resulted in a significant decrease in *P. kerathurus* stock abundance, reflected in declining annual landings in Spain, Italy and Amvrakikos Gulf (Ionian Sea, Greece) (Rodríguez 1977; Scordella and Lumare 2001; Conides et al. 2006). Therefore, in order to strengthen wild stocks and thereby increase catches, restocking could be a successful strategy (Cannizzaro et al. 2011a, b; Vitale et al. 2013). Indeed, in the case of species with a limited home range distribution, like the European lobster *Homarus gammarus*, the yield from released specimens (YPR) was high despite the expensive cost of juvenile production (e.g. Bannister et al. 1994; Agnalt et al. 2004; Agnalt et al. 2008; Schmalenbach et al. 2011; Carere et al. 2015; Aspaas et al. 2016). On the other hand, production of penaeid prawns, which had a lower YPR, could not be augmented without a huge number of released juveniles and maintenance of nursery habitats (Hamasaki and Kitada 2008).

In this paper we report a restocking trial and an assessment of the effectiveness of different diets on post-larval growth and survival. *P. kerathurus* is one of the less studied penaeid species, as it is difficult to culture due to high mortality, mainly at early post-larval stages. This critical phase occurs during the transition from pelagic to benthic life (Rodríguez 1985). Our experiment aimed at identifying a diet, which could allow rapid growth, decrease the costs associated with a longer maintenance of the post-larvae in the hatchery and reduce the natural mortality related to prawn size (Wang et Haywood 1999) optimising production efficiency, a better restocking efficacy and eventually a better YPR. Indeed, in aquaculture farms, feed represent at least 50% of total production

costs (Bender et al., 2004). For this purpose, PL10 post-larvae from the same female were kept in the hatchery for 8 weeks and allocated to four different diets (frozen and artificial).

5.2 Methods

5.2.1 Restocking trial

A restocking trial on *P. kerathurus* was carried out in 2015 along the coastal area of northern Lazio, Italy (Fig. 5.1) (42,0°N 11,8°E – 42,3°N 11.6°E). Breeders from the same area were caught by trammel net, and stage IV (mature) and III (partially spawned) (Primavera 1985) females with spermatophore in the thelycum (Tab. I) were selected and transported to the Ichthyogenic Experimental Marine Centre (CISMAR) hatchery in Tarquinia (Viterbo, Italy). As eggs began to hatch, the larvae, at nauplius stage, were fed three species of microalgae (*Isochrysis galbana*, *Nannochloropsis oculata*, *Chaetoceros calcitrans*, Ciani et al. 2018) and when they reached the mysis I stage the diet was integrated with freshly hatched *Artemia franciscana* nauplii. About 15 days post hatching, the larvae going beyond mysis III stage were considered post-larvae. At PL10 stage (10 days old post-larvae), the diet was also integrated with artificial feed made up of fish meal (salmon, halibut and rockfish), seafood meal (krill and shrimp), wheat meal and vitamins (A, D3, E, B12). The post-larvae at PL20 stage were lowered into the sea by scuba divers and released on a shallow sandy bottom. Five mature females out of 20 released 460.200 eggs, from which 118.922 nauplii hatched (29%). Only about 10.000 specimens reached the PL20 sub-stage and were used for restocking (Tab. I). According to Hamasaki (2006), probably this number of specimens released is still too low to allow a lasting effect on the wild stock population.



Fig. 5.1 Map of the restocking area (top).

Tab. I Broodstock, ovarian maturation stage, weight in grams, number of spawned eggs, number of hatched eggs (right).

Female	Stage	Weight (g)	Spawned	Hatched
1	IV	34,5	/	/
2	III	65	/	/
3	IV	66,2	18000	42%
4	III	19,2	/	/
5	IV	32,5	/	/
6	IV	27,6	46000	39%
7	III	62,4	/	/
8	IV	44	230400	26%
9	IV	38,9	/	/
10	IV	77,8	/	/
11	IV	35,5	/	/
12	III	31,5	/	/
13	IV	26,4	/	/
14	IV	78,2	124000	20%
15	IV	42,2	/	/
16	III	46,3	41800	18%
17	III	23,6	/	/
18	IV	34	/	/
19	III	22,5	/	/
20	IV	49,8	/	/

5.2.2. Growth experiment

A total of 60 PL10 post-larvae fed according to Ciani (2018) using *Chaetoceros calcitrans* microalgae and hatched from the same female, were kept in floating grids (Fig. 5.2) in a tank filled with 35‰ filtered seawater at 23°C. Water quality was guaranteed by a closed circuit equipped with a mechanical, biological filter, a skimmer and a U.V. sterilization system. The grids were divided into numbered cells, each housing a single post-larva in order to avoid cannibalism during the moult. Four different treatment groups were randomly formed (15 specimens each) each receiving a different diet: frozen mussels; frozen mysis; artificial wet mash containing salmon meal, maize meal, albumen, soy lecithin and astaxanthin; dry pellets (purchased by Omega Sea) containing fish meal (salmon, halibut and rockfish), seafood meal (krill and shrimp), wheat meal, astaxanthin, zeaxanthin, soy lecithin and vitamins (A, D3, E, B12) (Tab. II). Grids were checked twice a day (morning and evening) to assure the presence of food *ad libitum*. The remaining food and excreta of post-larvae were siphoned each day. The initial length (mm) at PL10 and final length (mm) at PL66 (after 8 weeks) of post-larvae

were measured from the tip of dorsal spine to the end of the telson furca (total body length, TL), taking pictures of each prawn and a scale ruler in the cell. Size was successively calculated through ImageJ, an open source image-processing program designed for scientific multidimensional images.



Fig. 5.2 Floating grid.

Tab. II Composition of wet mash and dry pellets diet.

WET MASH		DRY PELLETS	
frozen copepods	10%	crude protein	42%
krill meal	5%	crude fat	11%
salmon meal	15%	crude fibre	2%
herring and anchovies meal	20%	moisture	8.5%
maize meal	15%	ash	8%
wheat meal	20%	phosphorus	0.5%
soy lecithin	12%	omega 3	1%
astaxanthin	50 mg/kg	omega 6	0.5%

5.3 Data analysis and results

Data on the restocking trial are reported in Table I. Growth increase (difference between PL 66 and PL10, data normally distributed) was analysed by a simple ANOVA model followed by Fisher's PLSD post-hoc with diet (4 levels) as between subjects factor. No statistical significance was present at PL10 among the different diet treatments upon the experimental allocation ($F_{3, 55} = 0.33$; $p = 0.80$). The effect of diet on growth at PL66 was highly significant ($F_{3, 42} = 14.21$; $p < 0.0001$, Fig. 5.3). Four out of the six post hoc comparisons were significant (all $ps < 0.004$). Results from PL fed mussel diet did not differ from PL fed pellet diet ($p = 0.55$) and mysis diet did not differ from wet mesh diet ($p = 0.07$). Data on survival were analysed by chi square test. Survival was higher with pellet diet ($93.3 \pm 0.0\%$), followed in order by wet mash diet ($80 \pm 0.0\%$), mysis diet ($73.3 \pm 0.0\%$) and finally by

mussel diet ($66.6 \pm 0.0\%$), but no significant differences emerged between diets (Fig. 5.4). The analyses were performed with Statview II (Abacus Concepts, CA, USA).

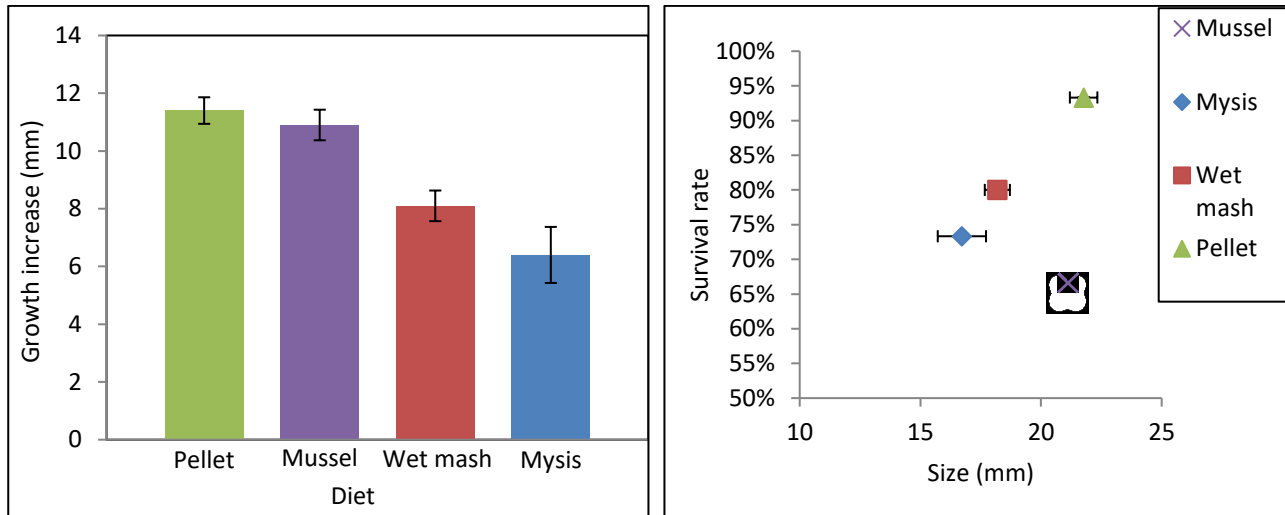


Fig. 5.3 Mean growth increase (mm) at PL66 (± 1 SE) at post-larvae PL66 for the four different diets: frozen mussels, frozen mysis, artificial wet mash, dry pellet (left).

Fig. 5.4 Mean size (± 1 SE) at post-larvae PL66 as a function of the survival rate for the four different diets: frozen mussels, frozen mysis, artificial wet mash, dry pellet (right).

5.4 Discussion

Synergies between aquaculture and fisheries management, through release of cultured juveniles, have been recognised for at least 30 years. Therefore, during these years, prawns breeding models were developed especially for species with a good growth rate, excellent resistance and high market value. The recent implementation of local management plans allows to support study on restocking or stock enhancement strategy for overexploited species not employed in aquaculture. These strategies should be tested systematically, verifying the actual increase of natural stocks and the profitability for the artisanal fishing activity. In 2015 about 10.000 *P. kerathurus* PL20 were released along the Lazio coastal area for this purpose.

Prawns are bottom-feeding, opportunistic omnivores, and consume a wide variety of foods depending on availability (Tidwell et al. 1997). However, their natural food preferences strongly depend on the

age. Phytoplankton is the primary food for larvae (Liao 1985), which become carnivorous, feeding on zooplankton, in a successive stage (Lavens et al. 2000), while *P. kerathurus* post-larvae and adults are omnivores, feeding on algae, crustaceans, molluscs and annelids (Ismael and New 2000; Karani et al. 2005, Prato et al. 2011). Among the natural foods for most penaeids, fresh mussel (*Mytilus edulis*) flesh is considered among the best, and is therefore largely used in nutritional studies, as a control diet (Faranda et al. 1982; Sedgwick 1979,1980; Fernandez and Puchal 1979) or as a major component of mixed diets (Sedgwick 1980; Forster and Beard 1974). Moreover, efficient feeds should be rich in protein, and the distribution of amino acids, especially the basic amino acids, should be similar to that of the prawn (Deshimaru and Shigeno 1972; Phillips and Brockway 1956), and mussels and mysis flesh have similar levels of amino acids. However, fresh mussels are not used for commercial rearing because of the high costs.

In this study, pellet diet was found to be the best for *P. kerathurus* post-larvae gain in size, while mussels too had a good response, although with a lower survival rate. On the other hand, wet mash diet showed a low mean size at PL66 post-larvae, but a good survival rate. The worse diet was the mysis. These preliminary results, although requiring replicas using different females, do not support previous studies with penaeid post-larvae by Faranda (1982) reporting a better survival on fresh mussels and mysis diets; probably, as suggested by James and Tong (1997), freezing may have deleteriously altered the flesh nutritional quality, perhaps reducing vitamin potency or altering the bioavailability of some critical micronutrient. Indeed, in *P. merguensis* the incremental introduction of vitamins into a frozen diet progressively reduced the extent of mortality and improved growth, moreover, when a mineral supplement was incorporated in addition to the vitamins, a further improvement was achieved (Sedgwick 1980). Wet mash diet was formulated adopting industrially available material of low price, mainly based on fish and vegetables flours, and it yielded a good rate on post-larvae survival but a low gain in size. The predominance of fish meal in the prawn feed never brought good results, probably because of deficient basic amino acids (Deshimaru and Shigeno 1972) or its low stability in water.

Absolute protein requirements appear considerably lower than those of *P. japonicus*. Deshimaru and Shigeno (1972) showed that the most rapid growth with this species occurred when test diets contained more than 60% crude protein (mussel and mysis diets), while in the present work no significant growth gains were recorded above 40% crude protein (pellet diet). Differences in protein intake may be associated with species differences in feeding habits. Examination of gut contents shows that *P. japonicus* is largely carnivorous while *P. karathurus* is more omnivorous in the wild (Ismael and New 2000; Karani et al. 2005, Prato et al. 2011). The early stage of *P. karathurus* post-larvae showed to have a developed gut, which is the main site for secretion of digestive enzymes during early developmental stages of penaeid prawns (Lovette and Felder 1990; Abubakr and Jones 1992). Indeed, they were able to readily digest artificial diets instead of live food which is highly digestible (New and Valenti 2000; Jones et al. 1993).

This experiment suggests that the caramote prawn post-larvae could be reared by feeding exclusively on an artificial diet, dry pellet, instead of conventional food such as mussel flesh, reducing costs related to live food. Moreover, as prawn diets represent the major contribution of pollutants in effluent water (Lawrence et al. 2001), and dietary protein is the main source of nitrogenous wastes in prawn culture systems (Moeckel et al., 2012), a diet containing lower protein levels could reduce its influence on water quality (Bechara et al. 2005; Wang et al. 2016).

Further research should be carried out to see if already the first stage of post-larvae (PL1) could be cultured using dry pellets instead of *Artemia* nauplii (which have a high price as cysts and are difficult to obtain as live or frozen feed) further reducing culture costs.

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6 Synthesis, conclusions and perspectives

In the last fifty years, increasing fishing efforts, pollution, loss of coastal habitats as well as unsustainable fishing practices have been pushing many fish and invertebrate stocks to collapse. Consequently, different global actions were undertaken in order to restore stocks and achieve healthy and productive oceans. The intensive exploitation of caramote prawn (*Penaeus kerathurus*), a benthonic coastal crustacean, resulted in a significant decrease in stock abundance (Rodríguez 1977; Scordella and Lumare 2001; Conides et al. 2006). The high demand of penaeid prawns stimulated, on one hand, the development of aquaculture activities, on the other hand, restocking actions to support artisanal fishery in several countries worldwide (Lumare 1984; Fushimi 2005; Hamasaki and Kitada 2006; Cannizzaro et al. 2011). Indeed, the practice of restocking can be considered a useful management measure, which matches the expectations of fishermen involved in artisanal fishing in the coastal areas. Therefore, a strategy of restocking could improve the activity of small-scale fisheries and, within an integrated coastal zone management, would contribute to the diversification of fishing effort by reducing fishing pressure on other overfished species with slower growth as the European lobster (*Hommarus gammarus*) or the spiny lobster (*Palinurus elephas*). Indeed, the fishing activity on lobsters is incompatible with that of the caramote prawn and the commercial value of the two species is basically the same (about 50 € per kg).

Identifying the best harvesting strategies and therefore knowing the right nutrition for the most delicate stages in prawn development (larvae and post-larvae) is the first step to optimize production in aquaculture and for an efficient restocking plan. The first part of this work was focused on finding a better algae based diet in terms of larval survival and growth, since due to a poor attitude of caramote prawn in being used for aquaculture purposes (low growth rate and limited temperature range), information about optimal diet and food regime of this prawn larvae and post-larvae were very scanty. The first results showed that diet of mixed micro-algae *Chaetoceros calcitrans* and *Isochrysis galbana* had better results for the larvae of *P. kerathurus* than single-alga diets. Indeed, as well as in other studies on penaeids (Becker 2004), survival and development were even better on a mixed algae

based diet, while, in this work, *I. galbana* alone always performed worse. Differences in the composition of fatty acids in the algal diets appear to be the cause, indeed *C. calcitrans* and *N. oculata* are characterised by significant concentrations of EPA (20:5n-3) that appear to induce a good growth. Instead high concentration of DHA (22:6n-3) are found in *I. galbana* (Volkman et al. 1989), the absence of this fatty acid in the diet seem related to a high mortality in *P. kerathurus* larvae.

A significant female effect on the survival of caramote prawn larvae was also found. Maternal effects are female non-genetic and genetic factors that contribute to offspring fitness (Green 2008), and as in other penaeids (Chu and Lui 1990; D'Souza 1999). Most of this variation may be due to some differences in the quality of the nauplii from different spawnings. Very few studies were carried out on maternal influences in decapod crustaceans (Giménez and Anger 2003; Darnell et al. 2009), despite the fact that this group includes large numbers of important fishery resources. Some works were carried out on *Homarus gammarus* suggesting that maternal size may have a positive effect on offspring survival (Jørstad et al. 2005). However, the mechanisms linking maternal influences on egg size to larval size and viability in crustaceans are still unclear. Certainly, recognizing the presence of maternal influences on *P. kerathurus* offspring, and further discriminating genetic and non-genetic factors, will greatly contribute to establish measures that optimize resources management and minimize potential impacts of fishing.

In the second part of the work, four different diets were tested on *P. kerathurus* post-larvae to identify a cheap diet, which combines rapid growth and good survival, essential for prawn culture in a controlled environment. A diet based on fresh mussels and frozen shrimps consistently gave good results (Wickins and Beard 1978), but it was considered too expensive (except as food for broodstock). Moreover, these and other wet diets have a rapid decomposition, which may create areas of deoxygenation. Mussel and mysis diets did not yield, as should have been expected, the best performance on *P. keraturus* post-larvae, probably because they were frozen and this could have affected the nutritional quality of the flesh. A diet of dry pellets with more than 40% of crude proteins was the best for growth and survival, showing a lower protein requirement. This suggests that

caramote post-larvae could be reared from PL10 with artificial feeds (the gut at that stage appears to be enough developed to digest dry pellets), reducing costs related to fresh or live food. Moreover, a diet with a lower protein content reduces nitrogen compounds ensuring a better water quality.

In summer 2015, a restocking trial was carried out along the coastal area of northern Lazio, Italy (the same area where the breeders were caught), releasing a total of 10,000 *P. kerathurus* PL20 on a shallow sandy bottom. Probably the number of specimens is still too low to allow a lasting effect on the wild stock population. Indeed, very large numbers of post-larvae are needed for an effective restocking plan, but even where hatcheries can produce millions of fit individuals at a reasonable cost, the releases will not have an economic impact on catches unless appropriate fishing regulations are issued (Doherty 1999; Bell et al. 2005).

On the basis of these results, further studies should be undertaken on other micro-algae rich in EPA and DHA type fatty acids during the larval stages of caramote prawn and the possibility of feeding very early stages of post-larvae with pellets should be verified. Moreover, although many works indicated that restocking or stock enhancement could be beneficial, assessing whether and to which extent releases of cultured post-larvae achieved their objective is essential to responsible and adaptive management. Faced with the difficulty of using a physical tag (e.g., moulting or juveniles and post-larvae small size animals) to tag large numbers of specimens, developing a genetic stock identification method would be appropriate. Rothlisberg et al. (1999) described some of the options for genetic tags of penaeid prawn, including three different types of DNA markers: mitochondrial (Benzie et al. 1993), microsatellites (Meadows et al. 2003) and amplified fragment length polymorphisms (AFLPs) (Moore et al. 1999). Unfortunately, this is not always straightforward for marine invertebrates because both tagging procedures and extensive sampling programs needed to retrieve tagged animals can be a significant cost during the research for, and application of, restocking and stock enhancement. Despite that, microsatellite DNA loci should be identified, developed and deployed to establish the genetic stock structure of *Penaeus kerathurus* and to evaluate their potential to ensure a discrimination between released and wild prawns.

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