We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

9

Applied Ecophysiology: An Integrative Form to Know How Culture Environment Modulates the Performance of Aquatic Species from an Energetic Point of View

Carlos Rosas[1], Cristina Pascual[1], Maite Mascaró[1], Paulina Gebauer[2], Ana Farias[3,4], Kurt Paschke[3] and Iker Uriarte[3,4]
[1]*Unidad multidisciplinaria de Docencia e Investigación, Fac. de Ciencias, UNAM, Puerto de abrigo s/n, Sisal, Yucatán*
[2]*Centro I-Mar. Universidad de los Lagos, Puerto Montt, Chile*
[3]*Instituto de Acuicultura, Universidad Austral de Chile, Sede Puerto Montt*
[4]*CIEN Austral*
[1]*México*
[2,3,4]*Chile*

1. Introduction

Ecological energetics as a part of ecophysiology, appears to have grown out of the Age of enlightenment and the concerns of the physiocrats, a group of economists who believed that the wealth of nations was derived solely from the value of "land agriculture" or "land development." Their theories originated in France, were most popular during the second half of the 18th century. Physiocracy is perhaps the first well-developed theory of economics (Danbom, 1979). The Age of Enlightenment (or simply the Enlightenment) is the era in Western philosophy, intellectual, scientific and cultural life, centered upon the 18th century, in which reason was advocated as the primary source for legitimacy and authority. It is also known as the Age of Reason. The enlightenment was a movement of science and reason. Ecological energetic began in the works of Podolinksy in the late 1800s, and subsequently was developed by the Soviet ecologist Stanchinskii, the Austro-American Alfred James Lotka, and American limnologists, Raymond Lindeman and George Evelyn Hutchinson. It underwent substantial development by H.T. Odum and was applied by system ecologists, and radiation ecologists to understand how the forcing factors modulate the ecosystem interactions (Weiner, 2000). Currently ecophysiology is a discipline that, in aquaculture, have been widely used to establish how the environment modulates the performance of animals in order to obtain the highest amount of biomass in the shortest possible time and cost. The use of physiological capacities of organisms to obtain biomass has been one of the basic premises of the application of ecophysiological studies to the production of aquatic organisms.

2. General aspects of applied ecophysiology

The physiological ecologist seeks to understand the organism in relation to its environment. Defining the environment in aquaculture is relatively easy because it is, in many cases, a relatively closed system with the exception of marine cages or extensive culture. Three basic concepts are involved in applied ecophysiology (1) perception, (2) distribution on the culture environment and (3) the environment. Different animals perceive their surroundings in different ways, and to some extent, physiological and behavioural responses are dependent on perception. There is always a danger that we will superimpose our own human perceptions on other species and environments. Aquaculture is plenty of procedures that farmers have been translated from agriculture to aquaculture with not always success and in so doing fail to appreciate the interaction between organism and culture environment. The aquatic environment is completely different from the terrestrial environment and thus the perception of animals is so different from that we have. Water is more dense than the terrestrial environment provoking that organisms have developed a series of adaptations to perceive smells, spaces and physical and chemical conditions completely different from those of terrestrial animals.

Distribution on the culture environment take into account that individuals have limited tolerance ranges of temperature, salinity and other physical and chemical factors. Thus, is a truism that populations of organisms not be found in abundance beyond the tolerance regions of most individuals. Physiological tolerance and functional morphology go a long distance toward predicting where a particular organism will not occur, but they often give little indication of how well or what the organism will be doing, whether it will occur at all, within its tolerance limits. Applied to aquaculture, ecophysiology tries to establish the tolerance and resistance of species to environmental variables in order to provide the best culture conditions and how must be the environment to obtain the maximum scope for growth. Aquaculture environment is one of the topics of applied ecophysiology because the structure of the environment, interaction between individuals, water chemical and physical characteristics, type of food, etc., affect the physiology of aquatic animals enhancing or limiting animal performance, and at the end the biomass production. In this context, seed density, behavior characteristics that enhance or reduce cannibalism, turbidity, ecological characteristics of the culture systems that include meio-fauna and vegetation, are analyzed.

A physiological response represents the sum of all cellular and biochemical reactions as influenced by the environment or the animal itself. For this reason, organisms are capable of reflecting any environment condition even before the effects are observed in the population and community level. In ecological energetics an energy budget equation is defined as the sum of the energy from food ingested, which is divided into metabolizable, egested and excreted energy. This energy will varied according to the effects of different extrinsic and intrinsic factors and therefore it is important to calculate the cost of production in terms of growth when considering total aquaculture activity. Thus, the animal production (P) or growth is represented by the difference between the absorbed energy and the energy lost in respiration and excretion, taking age, sex, and body type into account. As with growth, other measures of biological productivity, such as work, egg production and body condition, will also affected by type of food or/and environmental characteristics. Thus, a satisfactory diet/environment in terms of the best efficiency will be needed in order to achieve optimal animal production.

This chapter will dedicated to show some examples that illustrate how applied ecophysiology have been solving some questions related with culture organisms and its consequences on production. In this chapter an integrative perspective related with immune condition is included taking into account that many of healthy problems observed on cultured animals are derived from immune problems provoked by inappropriate culture environments. Finally, some aspects related with experimental designs that must be considered in studying of applied ecophysiology are proposed. To exemplify, two species groups were formed: molluscs and crustaceans. Into the mollusks, examples with bivalves, gastropods and cephalopods are presented. Into the crustacean, examples with crabs and shrimp are used to illustrate how applied ecophysiology can improve aquaculture. In the case of fish a new encyclopaedia of fish physiology (from genome to environment) was published recently (Farrell, 2011). For that reason fish were not included in this chapter.

3. Molluscs ecophysiology

3.1 The case of bivalve *Argopecten purpuratus*

Bivalve filter feeders are capable of ingesting living and inert particles suspended in the water column that are responsible for the energetic input. In some species was demonstrated that detritus may contribute to the diet during periods in which the environmental offering of phytoplankton is unable to satisfy their energetic requirements. Although organic materials from discarded feed and faeces from salmon culture contributes to the diet of pectinids maintained in culture in southern Chile, phytoplankton is the main nutrient source for bivalves aquaculture (Farias & Uriarte, 2006). As pointed out, the shell growth and biochemical composition of larvae give clear indications about changes in the quality of the environment that are basic to determine how water nutrients and phytoplankton modulates the biomass production of bivalves (Ferreiro et al., 1990).

Studying pectinid *A. purpuratus* several ecophysiological parameters were investigated to find the best conditions to cultivate this bivalve species. In a first step biochemical composition of larvae and spat was used to evaluate the energy metabolism and the nutritional condition of hatchery reared bivalves (Farías et al., 1998). Adults from commercial long lines were stimulated to spawn using temperature shock and total lipids, soluble protein and total carbohydrates of eggs, larvae and spat were evaluated.

From that result was evident that lipids were the main source of energy while protein is deposited into the biomass. At the same time, an increment on carbohydrates was observed suggesting that the metabolic pathways related with transformation of lipids into glucose could be activated during larvae development and putting in evidence that quality of food used during larvae culture greatly influenced the storing of reserves and the survival of the resulting spat (Fig. 1).

These results were confirmed latter when the effect of dietary protein content on biochemical composition of postlarvae, spat and gonadal development of *A. purpuratus* was tested (Uriarte & Farías, 1999; Farías & Uriarte, 2001). From that results it was evident that pectinid, and other bivalves production depends on the quality of the diet and the environment in which they inhabit (Fig. 2). Results on reproductive conditioning of adults showed that feed quality affect the quality of eggs depending of the species, so for *A. purpuratus* an increase in micro algal protein produce an increase in fertility and growth rate

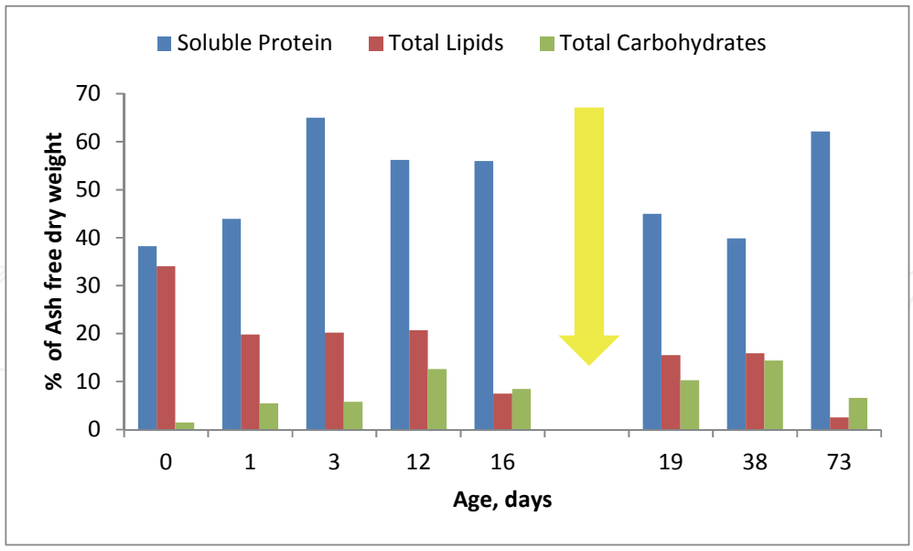


Fig. 1. Biochemical composition changes during larvae and postlarvae development of *A. purpuratus*. Arrow indicates metamorphosis.

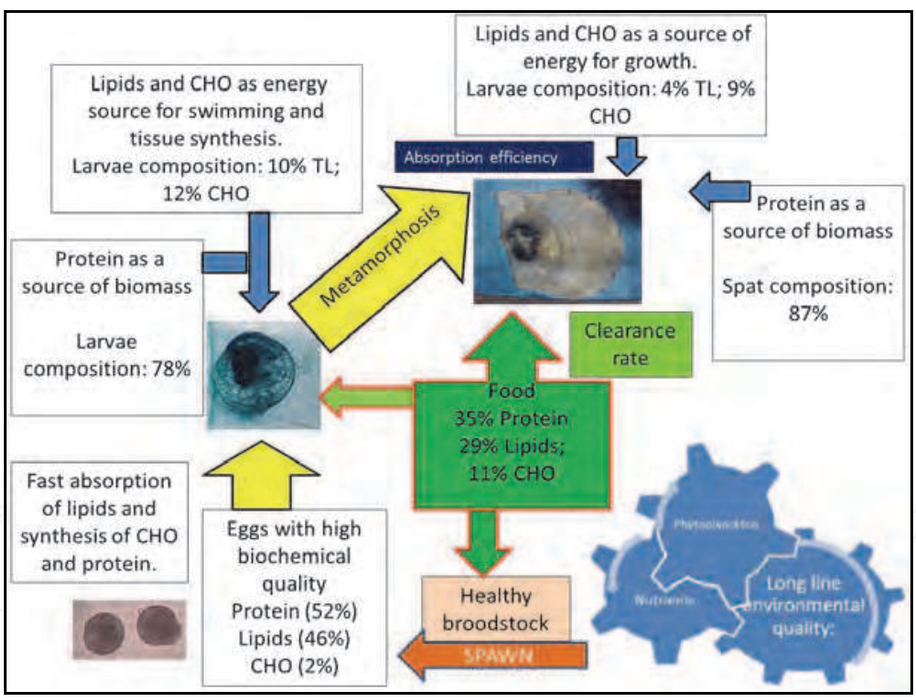


Fig. 2. Nutrient flows and biochemical pathways of planktonic larvae, spat and broodstock of *A. purpuratus* under culture condition. High quality eggs from healthy broodstock favors the use of lipids as a source of energy and synthesis of glycogen, while protein are used to protein deposition . After metamorphosis, carbohydrates and lipids are used mainly as a source of energy for maintenance and to accumulate protein via biomass production of spat. Food quality, measured as protein content, and environmental quality (Temperature, salinity, dissolved oxygen, turbidity etc.) determine the growth rate of bivalves, being the clearance rate and energy absorption key of culture success.

of larvae while in *Crassotrea gigas* non effect in fecundity was observed but eggs showed the highest lipid reserves and larvae showed highest survival. The scope for growth and reproductive conditioning of *A. purpuratus* is the product of the combined effect of high clearance rate and absorption efficiency that animals showed when fed high protein levels, indicating that phytoplankton abundance and composition will determine culture success (Farías & Uriarte, 2001).

The cost of the feeding depends as much on the processes pre- as post-ingestion and its modelling allows predicting, quantitatively, the growth of the bivalves based on simple but robust relationships between the conducts of feeding and the quality and amount of the food available in the environment. Although the importance of food quality and environment have been acknowledge, variability of the environmental quality and food remains as the principal concern between mussel and bivalve farmers, that depends on the natural food and environmental quality to maintain long line marine bivalves production (Lovatelli et al., 2007).

3.2 The case of gastropod *Haliotis fulgens* and *H. rufescens*

From the 130 species Halliotidae reported worldwide, only 14 species have been commercially exploited, either from fishery or through aquaculture. Since the commercial overfishing has severely depleted most populations of abalone, their aquaculture is replacing great part of the demand of these species. For abalone or any other cultured species, feed composition and ingestion rate are between most important factors to consider to predicting biomass production. In this sense, the study of *H. fulgens* have been used as an example to understand how the type of diet modulates the use and destination of the ingested energy, in this particular case, in attempt to optimize the use of macro algae or balanced foods.

Energy budget is a basic tool to evaluate the effect of type of food or other factors on physiological condition of aquatic animals. Using this model and considering different sizes of organisms, it is possible to obtain enough information to estimate their energy needs, and in a particular condition, predict the energetic costs associated with a particular environmental condition or type of food. In *H. fulgens* the energetic balance of animals between 0.1 to 2.5g was obtained in animals maintained in optimal conditions (Table 1) (Farías et al., 2003).

Relationship	Type of model	a	b	r^2
Dry body weight (g) DBW vs Total lenght (mm) L	$DBW = a\ L^b$	0.00001	3.08	0.98
Wet body weight (g) WBW vs Live weight (g) LW	WBW = a LW-b	0.15	0.63	0.99
Dry body weight (g) DBW vs Live weight (g) LW	DBW = a LW+b	0.02	0.11	0.98
Ingestion rate (mg day^{-1}) I vs dry body weight (g) DBW	$I = a\ DBW^b$	24.3	0.6	0.69
Absorption rate (mg day^{-1}) Ab vs dry body weight (g) DBW	$Ab = a\ DBW^b$	21.01	0.6	0.69
Respiration rate ml O_2 day^{-1} R vs dry body weight (g) DBW	$R = a\ DBW^b$	12.01	12	0.69
Nitrogen excretion (µmol N-NH_4 day^{-1}) U vs dry body weight (g) DBW	$U = a\ DBW^b$	43.6	0.85	0.63

Table 1. Relationship between morphometric characteristics and physiological responses of different sized green abalone *H. fulgens*.

With these equations it is possible to calculate how much of energy abalones needs per mg of biomass, how much of energy could be obtained, as a scope for growth per joule of ingested food (Table 2) and at the end the quantity of food that is needed to produce 1g of living abalone.

Physiological response		joules day^{-1} mg^{-1}	%
Ingestion rate	I	457	100
Feces	H	62	14
Absorption rate	Ab	395	
Nitrogen excretion rate	U	16	4
Respiration rate	R	172	38
Scope for growth	SFG	207	45

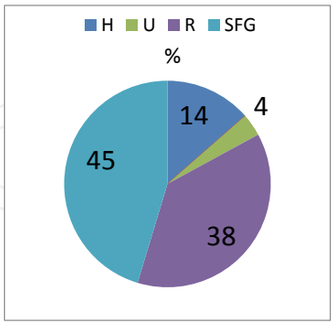


Table 2. Energetic balance obtained on optimal culture conditions of *H. fulgens*. Values obtained from equations derived from table 1. Circle indicates the proportion (%) of ingested energy (100%) that is channeled to each physiological response.

To make the calculations it is necessary to consider that 1g living abalone have 0.13g of dry weight biomass DBW (Table 1), that food have 18000 joules g^{-1} (Farias et al., 2003) and that scope for growth obtained was around 207 joules day^{-1} mg^{-1} DBW (Table 2). Following the next steps:

1. Joules day^{-1} needed to produce 1g living abalone =

$$\frac{(130 \text{ mg DBW of abalone contained into 1g living weight}) \times (207 \text{ joules day}^{-1}\text{mg}^{-1}\text{DBW})}{1 \text{ mg DBW}}$$

$$= 35{,}100 \text{ joules day}^{-1}\text{g}^{-1}\text{LW}$$

2. If 1g of food have a content of 18,000 joules g^{-1}, but only 45% of it (8,100 joules g^{-1}) are really converted in biomass, then the quantity of food required to produce 1g of living abalone will be:

Food to produce 1g of living abalone = 35.1 kjoules day^{-1} g^{-1}/8.1 kjoules g^{-1} = 4.3g or 4.3kg of food for 1kg of abalone. With these models it is possible to help farmers to calculate not only biomass production levels but cost of that biomass production.

In a more generalized application of energetic balance results it is possible estimate the food needed to produce other abalone species as *H. rufescens* (Hernández et al., 2009). In that case formulated diet with 4.15 kjoules g^{-1} wet weight were compared with two macro algae with 2.7(*Porphyra columbiana*) and 0.92(*Macrocystis pyrifera*) kjoules g^{-1} wet weight energy contents, respectively. At the light of that energy contents and assuming the same conversion efficiency of *H. fulgens* (45%) it is possible to observe that to produce 1kg of abalone with macro algae will be necessary to use 4.15kg of formulated food, 28.9kg of *P. columbiana* and 84 kg *M. pyrifera*, both given as a fresh food. Results of the *H. rufescens* assay showed that abalones fed with macro algae grew 120% and 40% higher than observed in animals fed formulated diet suggesting that macro algae could be used to cultivate abalones instead of formulated diet. However natural production of macro algae

in high quantities that could be demanded to produce biomass could be a limit for the growth abalone culture.

3.3 The case of cephalopods *Octopus maya*, *Enteroctopus megalocyathus* and *O. vulgaris*

Of cephalopods, octopuses are considered economically interesting species for aquaculture. Landings in this area have been steadily decreasing since the 90's, which has led to increase the demand for octopuses and thus the technological-scientific efforts to rear them. Rearing is defined as the development of juveniles from an egg or paralarva with the ability to reach a second stage (Boletzky, 2003). Many cephalopods have been subject of several studies in captivity intended to investigate behavioural aspects (Hanlon & Wolterding, 1989; Hochner et al., 2006), used as models in neurophysiology studies (Flores, 1983; Wollesen et al., 2009), in predator-prey relationships (Villanueva, 1993; Scheel, 2002; Smith, 2003) or to provide live specimens for aquariums (Summer & McMahon, 1970; Bradley, 1974; Anderson & Wood, 2001). In the last years, many studies related with octopus and sepia culture have been putting in evidence the increased interest in their culture (for reviews see) (Sykes et al., 2006; Iglesias et al., 2007; Uriarte et al., 2011). Octopus are semelparous, that means that females spawn once in their life and die soon after the eggs hatch (Hanlon et al., 1991).The life span of the most of cephalopods species is short varying from about 6 months in small species to between one to three years in larger ones (Villanueva & Norman, 2008). Recent works show the very fast and high rates of food consumption, food conversion and growth of octopus. Most of our knowledge of octopus energy budget comes from studies of few species potentially due to their fisheries value and their potential as culture species.

Octopus vulgaris is by far the most studied between octopus species. At the date there are many investigations related with some aspects of energy budget. To exemplify the use of ecophysiological studies on octopus culture there are several studies in which octopuses were fed squid and tested at different experimental temperatures (Katsanevakis et al., 2005; Miliou et al., 2005). Katsanevakis et al. (2005) tested the effects of temperature and body weight on respiratory metabolism of juveniles of *O. vulgaris*. Using data obtained on that study a surface plot was constructed using a quadratic model for routine metabolism (Rrout) expressed as joules per day (Fig. 3).

That model was constructed using an interval of octopus living weight between 0.1 to 1kg and a sea water temperature between 13 to 28°C. With this information we can now calculate how much energy will be metabolized if octopuses of 0.1kg are cultivated at 15°C and in consequence how much food should be used to cover that energetic demand: Using the model:

$$\text{Rrout} = 19403.7-[15.3\ (0.1\text{kg})] - [2040.6(15°\text{C})] - [0.003\ (0.1\text{kg})^2] + [2.4\ (0.1\text{kg} \times 15°\text{C})] + [52.2\ (15°\text{C})^2] = 541.2 \text{ joules day}^{-1}$$

To validate that model, we re-calculated the Rrout value obtained with last equation but using the living weight of octopuses used in other studies where Rrout was also measured. To do that we used weight values from García-Garrido et al. (2011) how made respirometric measurements of *O. vulgaris* (850g) fed squid at 15°C. The Rrout found by authors in that study was 671 joules day^{-1}, close to that obtained if the quadratic model is applied (554.4 joules day^{-1}). That concordance suggests that the quadratic model calculated now gave comparable data with other experimentally obtained (García-Garrido et al., 2011).

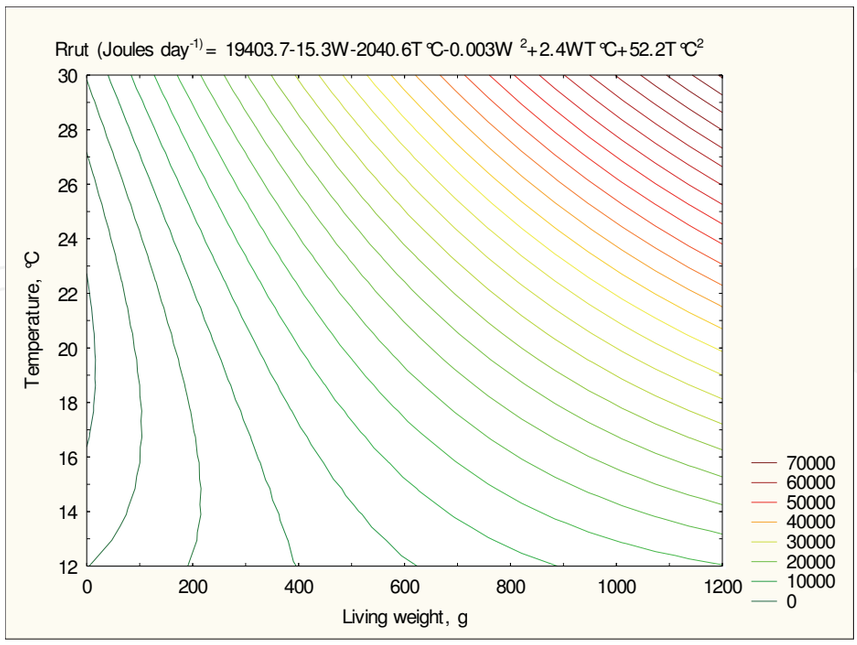


Fig. 3. Surface plot derived from a quadratic model to describe the relationship between Respiratory metabolism (joules day^{-1}) (Rrout = Routine metabolism; Rsda = Specific dynamic action metabolism; Rtot = Total metabolism) calculated for animals between 0.1 to 1kg living weight. (Data from Kasanevakis et al., 2005)

But, not always the models obtained give results applied at all mainly because the experimental procedures are not always are made in comparable conditions and depends on the form in which a determined factor affect the physiological response of animals. For example, using a model proposed by (Miliou et al., 2005) to evaluate the combined effect of temperature and living weight on feeding rate of *O. vulgaris* (Feeding rate g day^{-1} = -4.4608 + 0.9272lnW + 0.1138T°C – 0.0026T°C^2) we calculated the feeding rate (g day^{-1}) for octopuses fed squid with different living (W) weight and exposed at different temperatures (Table 3).

In their research, Miliou et al. (2005) feed octopuses with squid at a rate of 3% living weight day^{-1}. When the equation is applied the proportion of food consumed by octopuses resulted closed to that food rate (Table 3) suggesting that the data obtained with equation increased with temperature and living weight in a constant form (Table 3), producing a constant feeding rate. Using newly the study of García-Garrido et al., (2011), where octopuses were fed squid at a ratio of 10% living weight day^{-1} we observed that the model cannot predict the real feeding rate obtained by authors, that in this case was 8% living weight, because for octopuses feeding rate, and in consequence the energy derived of food, is modulated by the quantity of food that is offered, and certainly 3% could not be an ad libitum ratio.

Other species of octopus have been studied in attempt to quantify the physiological condition of octopus in similar conditions; a study was done comparing energetic balance of two species: *Enteroctopus megalociathus* (living at 10°C) and *O. maya* (living at 28°C) (Farias et al., 2009). The scope for growth of both species was calculated as: P = I – (R + H + U), and values of P of 522 and 358 joules day^{-1} g^{-1} were obtained of *E. megalociathus* and *O. maya,* respectively (Fig. 4).

		Feeding rate	
Living weight,g	Temperature, ℃	g day^{-1}	% living weight
200	15	4.8	2.4
	20	5.4	2.7
	25	5.3	2.7
400	15	9.2	2.3
	20	10.3	2.6
	25	10.1	2.5
600	15	13.4	2.2
	20	15.0	2.5
	25	14.7	2.5
800	15	17.4	2.2
	20	19.6	2.4
	25	19.2	2.4
1000	15	21.5	2.1
	20	24.0	2.4
	25	23.7	2.4

Table 3. Relationship between living weight (g) and temperature (°C) on feeding rate of *Octopus vulgaris* fed squid. Data obtained from the equation: Feeding rate g day^{-1} = -4.4608 + 0.9272lnW + 0.1138T°C – 0.0026T°C^2) (Miliou et al., 2005)

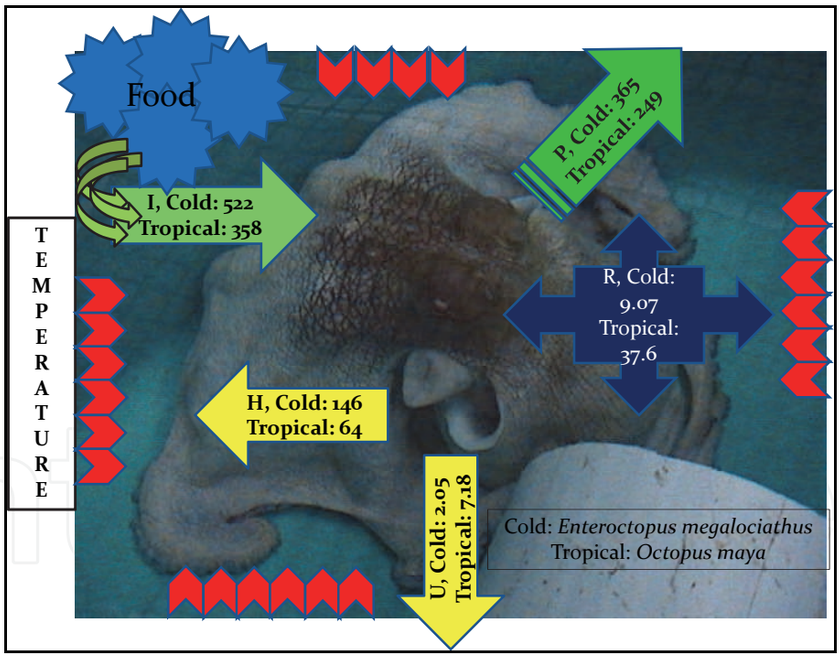


Fig. 4. The energy balance between species inhabiting cold (*E. megalociathus)* and tropical environments *(O. maya)*, showed that the scope for growth (P) of *E. megalocyathus* was 1.5 times higher than that of *O. maya*. In that study was demonstrated that cold-water cephalopod species could be more efficient than tropical species, a fact that should be considered at the time of culture.

That study shows also that each octopod species is adapted to use ingested energy in a different way as a consequence of the specific forces in each particular ecosystem: *O. maya*

loses more energy through ammonia excretion than *E. megalocyathus*, suggesting different physiological mechanisms for using the ingested protein (Table 4).

	a	b	R^2
Oxygen consumption, $mgO_2\ h^{-1}\ animal^{-1}$			
Enteroctopus megalociathus	0.2	0.71	0.82
Octopus maya	0.93	0.69	0.93
Ammonia excretion, $mgN\text{-}NH_3\ h^{-1}\ animal^{-1}$			
Enteroctopus megalociathus	0.32	0.37	0.53
Octopus maya	0.75	0.43	0.76
Ingestion rate, $g\ day^{-1}\ animal^{-1}$			
Enteroctopus megalociathus	0.19	0.67	0.95
Octopus maya	0.07	0.82	0.95

Table 4. Equation parameters ($Y = a\ W^b$) of oxygen consumption, ammonia excretion and ingestion rate of *Enteroctopus megalociathus* and *O. maya*. (Data from Farías et al., 2009).

4. Ecophysiology of crustacean

Between crustacean shrimp, crab and lobster has been deeply studied from an ecophysiological point of view. Crustaceans live in a wide variety of environments including fresh water, salty water and sea water. Crustaceans can be finding in holes in earth living with a little bit of humidity or in the crown of palms, several meters above the ground. Crustacean aquaculture has been growing in the last decades being the shrimp culture the most important activity. The world production of crustaceans was 5 million tons in 2008 being shrimp culture 73.3% of the total production (FAO, 2011). Today the white leg shrimp *Litopenaeus vannamei* is the most important species for aquaculture. *Litopenaeus vannamei* was introduced in China where its cultivation shifted to that of *Penaeus monodon*. Ecophysiological studies of shrimp have been important to define culture environments mainly when this species is mostly cultivated in salty water, where joint with diluted environment, animals are exposed to high temperatures, wide oxygen variations and nitrogen waste products.

4.1 Effect of salinity on shrimp physiological condition

White shrimp, *Litopenaeus vannamei*, the most cultivated shrimp species in America, grows well at salinities ranging from freshwater to hyper saline waters. Although *L. vannamei* tolerates broad salinity ranges, values for optimal growth have not yet been determined, and studies up to date show contradictory results. Whilst studies conducted under laboratory conditions have shown that maximal growth occurs between 5 and 15 UPS (Bray et al.*(*1994); Boyd, 1995; Rosas et al., 2001a), other studies have reported maximal growth at salinities above 35 UPS (Ponce-Palafox et al., 1997; Decamp et al., 2003). Recent studies have demonstrated a close relationship between nutrition and environmental characteristics at which shrimp are cultivated. This is because dietary levels of carbohydrates, lipids and proteins largely determine the shrimps capacity to respond to changes in the ionic composition of culture water.

4.1.1 Growth and survival

Salinity affects the distribution of a variety of estuarine and marine organisms. Some marine species, such as shrimps, have life cycles that include an estuarine phase. Since the 1950´s, there have been reports on congregations of juvenile shrimps at low salinities, whilst adults reproduced under strict marine conditions. It has also been observed that larval phases inhabit oceanic waters, where they develop and moult into postlarvae. Through certain chemo tactile mechanisms and water currents, postlarvae are capable of moving towards estuaries and coastal lagoons, where they recruit as early juveniles. It is in these habitats, with strong salinity variations, where some shrimp species are capable of colonizing more diluted environments than others. For example, studies carried out in the 1960´s by Mac Farland & Lee, (1963) showed that juvenile *L. setiferus* are better adapted to tolerate low salinities than juvenile *Farfantepenaeus aztecus*. Similarly, Mair, (1980) observed that amongst *L. stylirostris, F. californiensis,* and *L. vannamei*, the latter is the species that best tolerates highly diluted environments in estuaries and costal lagoons, and is therefore capable of inhabiting areas restricted to the other two species. This ecophysiological trait allows *L. vannamei* to reduce ecological pressures of competition for space and food, both strong limiting resources in these habitats. In an experimental study, Mair, (1980) observed that *L. vannamei* postlarvae and early juveniles placed in an experimental salinity gradient preferred salinities from 3-6 UPS, whereas *L. stylirostris* and *F. californiensis* preferred salinities from 32-35 UPS and 9-26 UPS, respectively.

The wide tolerance of *L. vannamei* to salinity has also been observed under culture conditions, both experimentally and within production facilities. Ogle et al. (1992) examined the effects of salinity on growth in *L. vannamei* postlarvae under different culture conditions. In that study the authors observed that tolerance to salinity is independent of postlarval (PL) age, showing that both PL8 and PL22 had better survival and growth at salinities ranging from 16 to 32 UPS. In that same study, it was demonstrated that tolerance to salinity increases as temperature declines from 30-16°C, since the highest survival and growth of postlarvae was obtained at salinities of 8 and 16 UPS at that temperature. Postlarvae can be acclimated to low salinities for production purposes. Recent studies have shown that a rate of salinity decline of 25% per hour is appropriate for postlarvae (PL10 to 4 UPS) and early juveniles (PL 20 to 1 UPS), demonstrating that this species is well adapted to highly diluted environments (McGraw et al., 2002). From these studies it is possible to conclude that *L. vannamei* postlarvae can tolerate broad salinity ranges, although shrimp between PL1 and PL10 are more sensible to low salinities that juveniles with more than 20 days after the last metamorphic moult (PL20).

In this study juvenile *L. vannamei* (initial mean weight 2.26g) were cultivated at 2, 4 and 8 UPS during 70 days in a semi-closed recirculating experimental system. Density was kept constant at 28 juveniles per m^2 in all salinity treatments. Results showed high survival rates in all treatments (98.7-100%). Mean final weight varied slightly between 19.0 -19.28g, whereas weekly growth rates varied between 1.67-1.7g. No significant differences in growth or survival amongst treatments were found, suggesting that *L. vannamei* can be cultivated at low salinities obtaining good survival and growth.

Bray et al., (1994) reported that juvenile *L. vannamei* positive to IHHN virus showed better growth exposed during 35 days at salinities of 5 and 15 UPS than at 25, 35 and 49 UPS. A

weekly growth of 2g was obtained at low salinities 5 and 15 UPS. Mean final weights of shrimp were 12g (5 UPS), 12.2g (15 UPS), 10.8g (25 UPS), 11.1g (35 UPS) y 9,8g (49 UPS). No significant differences in mean final weight were found amongst low salinity treatments (5 and 15 UPS), but these were both significantly higher than that obtained at 49 UPS. Survival values were statistically similar amongst all treatments. A second group of juveniles with mean initial weights of 2.2g were infected with the IHHN virus, and maintained at 25 and 49 UPS. Their growth and survival was then compared with those of shrimp of the first group kept at those salinities. Results showed that growth of infected shrimp was similar to that obtained in the previous trial. In addition, there was a significant interaction between shrimp group and salinity, indicating that the group infected with IHHN grew less at high salinities. Mean final weights in the second trial were 10.3g (25 UPS) and 8.5g (49 UPS). Survival was also statistically similar for infected shrimp kept under the two salinity treatments.

In a more recent study Ponce-Palafox et al., (1997) reported that optimum growth and survival for juvenile *L. vannamei* were between 33 and 40 UPS and 28 and 30°C. Besides growth and survival of *L. vannamei* postlarvae were measured at 20, 25, 30, and 35ºC and salinities of 20, 30, 35, 40 and 50 UPS. Groups of 30 individuals with three replicates were used in each temperature and salinity combination. Results clearly showed that juveniles of this specie survive best at 20 and 30ºC and at salinities higher than 20 UPS. Shrimp growth was highest at temperatures between 25 and 35ºC, with small differences amongst salinity treatments. Rosas et al.,(2001b) observed that better growth of juvenile *L. vannamei* can be attained when shrimp are kept at 15 UPS than at salinities of 35 UPS or more. Although much research on juvenile *L. vannamei* culture has been conducted, there is yet no decisive demonstration of the optimal salinity range. Whilst some researchers report that salinities lower than 25 UPS promote growth, others confirm that salinities higher than 35 UPS result best for this species. Combinations of salinity and temperature, as well as the type and quality of food appear to be determining factors to establish optimum salinity levels. Other shrimp species have also grown best at estuarine salinities

Chen et al., (1992) observed that *P. chinensis* kept at 25-31°C showed best growth and survival at salinities between 10 and 20 UPS. In addition (Staples & Heales, 1991) report best survival and growth in *P. merguiensis* kept under a combination of 20°C and 20 UPS. Rosas et al., (1999) showed that juvenile *L. vannamei* are energetically more efficient when exposed at salinities of 15 UPS than those lower (10 y 5 UPS) or higher (20, 25 y 30 UPS) than 15 UPS. In another study Brito et al., (2000) showed that juvenile *F. brasiliensis* have low tolerance to salinities lower than 25 UPS, below which shrimp stop growing and die. The authors explain that postlarvae and juveniles of this species occur in the southern Gulf of Mexico and Caribbean Sea, where they are rarely exposed to salinities lower than these. Rosas et al., (1999) found that salinity affects shrimp growth and survival differentially depending on postlarval age. These authors observed that optimal salinity for PL 10 lies between 30 and 35 UPS, whereas optimal salinity for PL 20 and older lies between 5 and 15 UPS. Optimal salinity ranges have been identified for other shrimp species. In general terms, species of the genus *Litopenaeus* are more tolerant to low salinities than those of the genus *Penaeus* or *Farfantepenaeus*. The former have optimal salinity ranges below 25 UPS, whilst the latter have optimal ranges at salinities above 30 UPS (Brito et al., 2000).

4.1.2 Relationship between salinity and food

Salinity is factor modulating the physiological state of shrimp. In costal environments where juveniles inhabit, estuaries and lagoons offer salinity conditions that fluctuate markedly both with tidal and seasonal variations. Penaeid shrimp have adapted to these changes taking maximum profit of food richness and protection found in these nursing areas. In order to survive and grow at the highest rates, shrimp have evolved through diverse physiological mechanisms that allow compensation for the marked changes in salinity. A decrease in water salinity results in a massive entry of water into the tissues by simple diffusion. In order to avoid extreme dilution and the consequent increment in cell volume, shrimp respond by: i) Reducing the concentration of ions dissolved in the cytoplasm. ii) Transporting amino acids from the cells to the blood, and then to the digestive gland. iii) Changing cell permeability at the gills. In crustaceans, ions transport has been extensively studied. Studies of Na, K and Cl transport as a result of exposure to diluted environments have shown that these ions play an active role in the reduction of cell volume by helping to keep osmotic pressure constant. However, because ions are small molecules with low osmotic power this mechanism is unsuccessful when shrimp are kept for long periods in diluted environments. A second mechanism has been described in which shrimp use the amino acid pool free in the cytoplasm to regulate cell volume. Amino acids are the principal component of proteins, and are abundant within shrimp cells. When a salinity change occurs, amino acids are transported towards the hemolymph where they are re-absorbed by the digestive gland. There, amino acids are stored as hemocyanin, as peptides for growth, or as proteins for the immune system. A recent study by Rosas et al.,(2002) includes a summary of the effects of dietary and protein and carbohydrate levels on the physiological and nutritional state of shrimp maintained at high and low salinities (Fig. 5).

It can be seen that CHO (carbohydrates) enter as starch and are transformed into glucose and glycogen. Laboratory studies have shown that starch breakup by amylase has a saturation point, indicating that shrimp capacity to breakup starch increases to a maximum level. At CHO dietary concentrations higher than 35%, the processing rate of amylase stays constant even with increasing CHO levels Rosas et al., 2000). Because in diluted environments amylase is less efficient, the saturation point of amylase has a larger effect under low than high salinities, (Rosas et al., 2001a). Another important aspect is the saturation of glycogen in the digestive gland (digestive gland) of shrimp fed with diets containing excessive CHO. Results of laboratory studies have shown that shrimp capacity to store glycogen is limited, and the digestive gland is rapidly saturated with levels of dietary inclusion higher than 30 %. When shrimp are kept at low salinities, this saturation has a larger effect, because it prevents adequate absorption of amino acids used to maintain internal osmotic pressure and cell volume Rosas et al., (2001a). These results have been interpreted taking into account that shrimp basically use CHO to form chitin, which in turn is the principal component of the exoskeleton. A glucose molecule and one ammonia radical product of the amino acid degradation form chitin. The combination of these products emphasizes the fact that chitin formation depends on CHO metabolism, which constitutes the center of penaeid shrimp nutrition.

The adaptations of shrimp cultivated in hypersaline environments have not yet been studied in depth. Studies conducted in our laboratory have shown that at high salinity the effects of dietary proteins and CHO operate in a different way than when at low salinity Pascual et al., (2003); Pascual et al., (2004a). From our results, it can be concluded that at high salinity

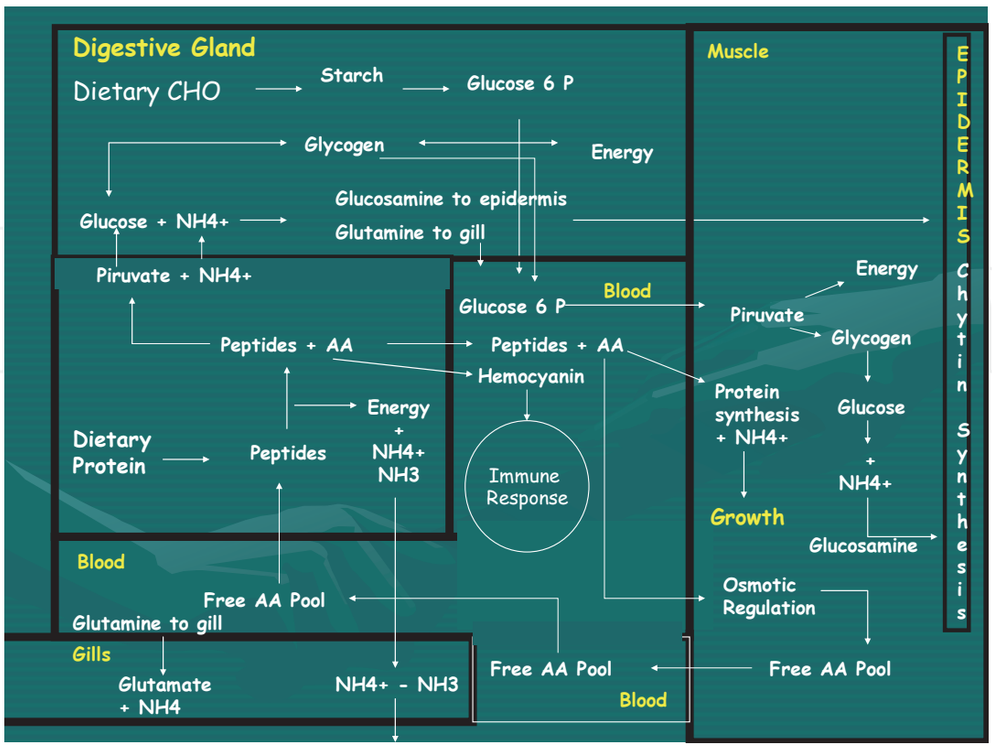


Fig. 5. Diagram showing a summary of the relationship between dietary proteins and carbohydrates and the metabolic pathways involved in the uptake of ingested nutrients.

shrimp may require lower protein levels than those they require at low salinity. This is because at concentrated environments ammonia produced by the degradation of dietary amino acids may be toxic. At low salinity degraded amino acids are transformed to ammonia and used for ion exchange, whilst at concentrated environments ammonia molecules are trapped in the blood and cannot be released as rapidly and efficient. Notwithstanding the high tolerance of crustaceans to high ammonia in blood Schmitt & Uglow, (1997), levels above 12 mg l^{-1} could inhibit growth, delay moult and reduce breathing capacity of shrimp Danford et al.(2001). High levels such as those can easily be attained when the diet contains more than 35% proteins and shrimp are kept at salinities higher than 40 UPS Rosas et al., (2000)

The effect of salinity on lipid metabolism in has neither been studied in depth. In a recent study the effect of salinity on shrimp survival, the activity of the Na/K bomb and lipids was examined Palacios et al., (2004). In that study 20 day old fed and unfed (3 and 24 h fasting) postlarvae were compared to demonstrate the effect of energy reserves on survival and osmoregulatory mechanisms. Activity of the Na^+-K^+ ATPase was 5 times higher in posterior gills than in anterior ones, a response associated with the osmoregulatory function of this organ. In addition, a non-significant increase in anhydrase activity was observed in postlarvae exposed for 20 days to 10 UPS. This is explained by the role of this enzyme in the hydration of CO_2 produced in respiration. Under low salinity conditions, the internal media dilutes and the output of Na^+ decreases. Na^+ in then exchanged for H^+ and Cl^- for HCO_3, provided by CO_2 through the carbonic anhydrase. In that same study total lipids in digestive gland were significantly lower in fasting than in fed PL_{20}, whereas triacyl glycerids

were statistically similar. This is probably due lipids in the digestive gland being used during the first 3 hours of fasting, allowing for animals to have sufficient energetic reserves to tackle the salinity change Rosas et al., (1995). This study concluded that movement of lipids to satisfy the energetic demand of osmoregulation could be operating in shrimp of this species.

4.2 Physiological condition and immune system of shrimp

The shrimp immune system has a solid protein base and hemocyanin plays an important role in its function. Recent studies have demonstrated that in addition to its multifunctional role (oxygen transporter, storage protein, carotenoids carrier, osmolite, ecdysone transporter) hemocyanin has a fungistatic Destoumieux et al.,(2001) and prophenol oxydase-like function Adachi et a*l.*, (2003). Proteins are also involved in recognizing foreign glucans through lipopolysaccharide binding protein (LPSBP) and β glucan binding protein (BGBP) Destoumieux et al., (2000); Vargas-Albores & Yepiz-Plascencia, (2000). A clotting protein (with the change of fibrinogens to fibrin) is involved in engulfing foreign invading organisms and prevents blood loss upon wounding Hall et al., (1999); Montaño-Pérez et al., (1999). Defense reactions in shrimp are often accompanied by melanization. Prophenoloxidase (ProPO)-activating system, mediated by hemocytes, is a zymogen of phenoloxidase (PO) enzyme that catalyzes both o-hydroxylation of monophenols and oxidation of phenols to quinones leading to synthesis of melanin Sritunyalucksana & Söderhall, (2000). Conversion of ProPO to PO occurs through a serine protease called prophenoloxidase-activating enzyme (ppA) regulated by another protein, a-2 macroglobulin, a trypsin inhibitor Perazzolo & Barracco, (1997). The innate immune response of shrimp also relies upon a production, in hemocytes, of antimicrobial peptides called peneidins that are active against a large range of pathogens essentially directed against Gram-positive bacteria via a strain-specific inhibition mechanism Destoumieux et al., (2000).

In order to reach effects of dietary protein level on energetic balance we probe two protein levels in a range of optimal reported levels of 15% and 40% (equivalent to 15 and 40g DP/kg body weight/day [g DP/kg BWd]) and one extremely low (5% equivalent to 5g DP/kg BWd) were used to feed juveniles for 50 days. Dietary protein level enhanced ingestion rate in shrimp fed 5g DP/kg BWd compared to shrimp fed 40g DP/ kg BWd, however, daily growth coefficient (DGC,%) of *L. vannamei* juveniles was high in shrimp fed 40g DP/kg BWd. An inverse relation between wastes (H+U) and dietary protein level was observed indicating that shrimp lose 81% of ingested energy when fed 5g DP/kg BWd and only 5.6% when fed 40g DP/kg BWd. A higher assimilation and production efficiency (P/As) was obtained when shrimp were fed 40g DP/kg BWd, than obtained in shrimp fed 15 or 5g DP/kg BWd. An increase in Oxy hemocyanin was observed with increasing dietary protein levels indicating that shrimp accumulated protein as hemocyanin. A reduction of hemocytes occurred when shrimp were fed sub-optimal dietary protein; same patron was observed in the respiratory burst. The compensatory mechanism used by *L. vannamei* to respond nutritional stress, sub-optimal dietary protein level (5 and 15g DP/kg BWd) induced not only a severe reduction in growth rate and assimilation efficiency but also in immune capacities Pascual et al., (2004).

In an attempt to know how the protein level modulates catabolism and its effects on the immune response, we studied juvenile *L. vannamei* that had been starved for varying period

after being conditioned on diet containing either maintenance or optimal dietary protein levels Pascual et al.,(2006). Juvenile shrimp were fed for 21 days on diets containing 5% and 40% dietary protein. Hemolymph metabolites (glucose, cholesterol, protein, acylglycerols, and lactate), hemocyanin, osmoregulatory capacity, digestive gland glycogen and lipids, and immune conditions (hemocytes concentration, phenoloxidase activity, respiratory burst: basal and activated) were evaluated and considered as initial condition. After that time, shrimp were starved for 21 days. A reduction in all physiological and immunological indicators was observed with starvation. The results demonstrate that shrimp are well adapted to tolerate food deprivation for some time but that this tolerance is closely related to its previous nutritional condition. In the case of shrimp fed 40% DPL, wet weight, nutritional and immune condition was significantly affected after 14 days of starvation. In shrimp previously fed 5% DP, tolerance to starving condition was limited to only a few days (7 days) as a result of low reserves of circulatory and mussel proteins. All these results demonstrate that dietary protein levels can governor the immune condition of shrimp through the management reserves metabolism.

Domestication is other important aspect to obtain better results during culture; however, artificial selection has important implication on physiological adaptations. Pascual et al., (2004) studied wild and seventh-generation cultivated shrimp to determine how size-based selection could alter the nutritional and immunological conditions of *Litopenaeus vannamei*. Wild juveniles and a sample of seventh-generation cultured shrimp were acclimated under identical conditions. During 55 days, shrimp were fed a high (HCHO: 44%) or a low (LCHO: 3%) carbohydrate diet. Wild shrimp showed a direct relation between dietary CHO and lactate, protein and hemocyte levels indicating that dietary CHO was used for protein synthesis via transamination pathways. In seventh-generation cultured shrimp these parameters were inversely proportional to dietary CHO level, indicating the capacity to synthesize protein from dietary CHO was repressed in cultured shrimp. Farmed shrimp showed a limited capacity to respond to LCHO diets demonstrating high protein dependence in their metabolism and immune response. These results demonstrate that during size-based breeding programs other metabolic process than CHO catabolism can be selected. The incapacity of shrimp to use dietary CHO could limit protein reduction of diets and limit the efforts of the shrimp industry to be ecologically and environmentally profitable.

Considering the results arising from a series of studies, Arena et al., (2003); Pascual et al., (2004 a and b), developed a conceptual model about how dietary components modulate the fate of energy intake, the nutritional status and immune system of juvenile *L. vannamei* (Fig. 6). The environment and genetic variability are the basis of the model, since as mentioned; the interaction between these elements affects the digestive capacity, the flow of energy, protein synthesis, osmoregulation, disease resistance and the degree homeostatic control.

Degradation of starch leads to glucose uptake, it is transported directly to the hemolymph or metabolized through glycolysis (**GL**); subsequently pyruvate through acetyl-coenzyme-A (**A-CoA**) can enter the Krebs cycle (**KC**) and continue with the respiratory chain (**RC**) to obtain energy (**ATP**). The digestive capacity of wild shrimp revealed the importance of taking advantage of dietary CHO, which can be associated with the various points of metabolic regulation of the glycolytic pathway. Nine of the ten essential amino acids can be synthesized from glucose, where glutamate provides the amino group. In the opposite

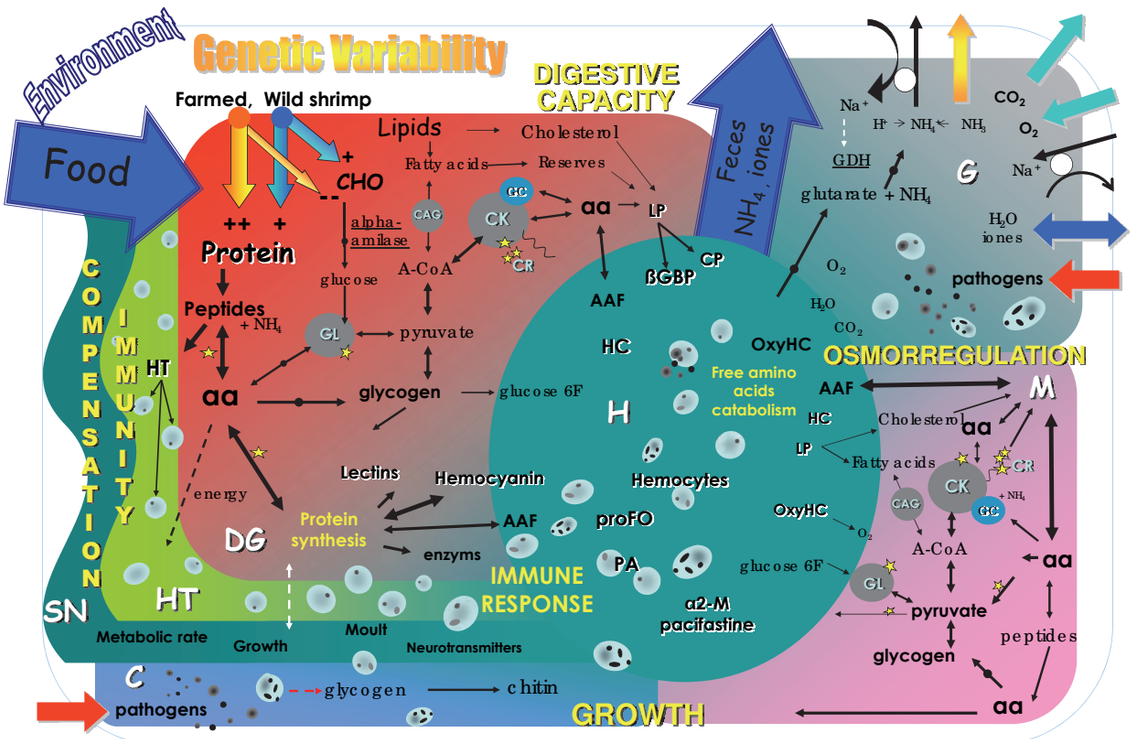


Fig. 6. Conceptual model: protein metabolism and homeostasis. The scheme includes the main organs and systems of juvenile shrimp: digestive gland, **GD**, gills, **G**; muscle, **M**; cuticle, **C**; nervous system, **SN**, hematopoietic tissue, **HT** and hemolymph, **H**. Genetic variability is the basis of the model as it affects the flow of energy from food chemistry to physiological processes. *Litopenaeus vannamei* juveniles grown in closed loop and sorted by size (F7, F25) have a limited capacity to respond to carbohydrate diets (CHO) demonstrating high protein dependence in their metabolism. The degradation of proteins generates peptides and amino acids (**aa**) that are oxidized or used to synthesize **glycogen**. The **aa** can also be used for the synthesis of digestive and metabolic enzymes, and immune proteins (such as prophenoloxidase, **ProPO**, lectins, **L**, lipoprotein recognition, **BGBP**, clothing protein, **CP**, antimicrobial peptides, **AP**; of regulation of the immune response; pacifastine and α2-macroglobulin, and hemocyanin, **H**, which seems to play a role in the immune response of shrimp, in addition to functioning as a storage protein in the hemolymph. Amino acids can also be transported to the hemolymph to form the pool of free amino acids (**FAA**), which can be used to generate muscle tissue.

direction, for example in conditions of nutritional stress, catabolism of glucogenic amino acids is the basis for the gluconeogenic route for CHO can be used as energy substrate. The cycle of fatty acids (**CAG**) is connected to the glycolytic pathway through **A-CoA**, a high concentration of **ATP** or **NADH** promotes the synthesis of fatty acids from the A-CoA. The ß-oxidation of lipids from food or reserves represents the reverse direction to generate metabolic energy.

Regulation of osmotic pressure is mainly associated with the Na-K ATPase, ion exchange, the catabolism of aa and the activity of glutamate dehydrogenase (**GDH**), which controls

the addition or removal of ammonia. Glutamate cycle (**GC**) is represented by a circle attached to KC. The amino group can be transferred to glutamine, which is less toxic than NH_4, so is involved in nitrogen excretion through the gills. The amino group can also be coupled to a carbohydrate to the formation of glucosamine for chitin formation. Therefore, growth and immunity of juveniles of *L. vannamei* are linked to domestication and protein metabolism.

The hematopoietic tissue and hemocytes are represented in the model under a deep interaction with the GD and the nervous system, since nutritional status and some hormones affect the rate of proliferation of hemocytes and thus the state of the immune response, because in the hemocytes are synthesized many of the immune effectors. On the other hand, the concentration of hemocytes in the epidermis during some stages of moult may be related to the release of glycogen for the synthesis of chitin, which is consistent with the ability of hemocytes to synthesize glycogen and store huge amounts (proportionately greater than content in the digestive gland). Also, the high amount of amino acids within the hemocytes could point to a possible release into the hemolymph associated with osmotic regulation and/ or energy demand Claybrook, (1983). Since the metabolic functions of hemocytes have not been demonstrated, in the model are marked with a dashed arrow.

4.3 Effect of temperature on energy losses during southern king crab larval development

Temperature is one of the most important environmental factors affecting every aspect of an organism's physiology, from the basic structures of the macromolecules that are responsible for catalysis and information processing to the rates at which chemical reactions occur Hochachka & Somero, (2002). Thus, energy acquisition, losses, allocation to maintenance and growth in any organism is affected by temperature. In heterotrophic organisms, the ingested food provides the energy input which is balanced with the main sources of energy losses i.e. bio deposition or faeces rejection, metabolism quantifiable by the oxygen consumption rate, and in less extent nitrogen excretion. The reminder energy is canalized to growth and/or reproduction. In a lecithotrophic (i.e. food independent) condition, the principal energy losses are related to respiration and nitrogen excretion.

The lithodids, commonly known as king crabs or stone crabs, inhabits high latitude cold waters of both hemispheres. Several species of king crabs represent valuable fisheries but commercial exploitation and environmental factors has dramatically declined the landings. In the southernmost part of South America (Chile and Argentina) *Lithodes santolla* (Molina), the southern king crab, is commercially exploited and severe fishing restrictions are necessary to protect the populations. Both circumstances increased the interest and expectative about the development of cultivation technologies for the southern king crab.

In order to establish larval rearing conditions, ecophysiological studies on *L. santolla* larvae have been carried out to define culture temperature based in the energy losses.

Early life-history stages tolerate low temperatures and their larval development is fully independent of food (lecithotrophic) Anger et al., (2004); Calcagno et al., (2004); Kattner et al., (2003); Lovrich et al., (2003); Thatje et al., (2003).

Our results showed that the development time fluctuate between 55 and 42 days approximately, at 9 and 15ºC, respectively. The sustained even increasing respiration rate throughout the zoeal stages is interpreted as a confirmation of lecithotrophic development, otherwise a drop in metabolic rate in starved larvae were expected (Fig. 7a). Megalopa showed at lower temperatures a decreasing respiration rate attributed to a behavioral change in the swimming activity, while at higher temperature the metabolic rate increased (Fig. 7a). A similar pattern was observed for the nitrogen excretion rate (Fig. 7b). The atomic proportion of consumed oxygen and excreted nitrogen (O:N) is considered as an estimator of the metabolized substratum; values between 3 and 16 would indicate protein metabolism, an O:N ratio between 50 and 60 corresponds to similar proportion of lipid and protein catabolism and higher values than 60 represents lipid metabolism Mayzaud & Conover, (1988). *L. santolla* larvae reared at 12ºC, in general showed a combined protein and lipid catabolism, but, at 9 and 15ºC the Zoea I showed an evident lipid metabolism (Fig. 7c). Respiration rate, independent to temperature, contributed principally on the energy losses, ranging between 93 and 96% at 9 and 15ºC. Although the shortest development time was at 15ºC, low survival suggests unsuitable rearing conditions. Indeed, the highest energy losses expressed as excretion and respiration rate (Figs. 7 a and b) occurred at 15ºC. Multiplying the energy losses of respiration and excretion rates by the respective developmental time, the cumulative energy losses were estimated. Similar energy losses during Zoea I and II were observed for the different temperatures, while an evident impact of higher temperature on the megalopa stage was observed (Fig 7d). The results suggest that elevated temperature ≥15ºC could generate a mismatch on the larval metabolism of *L. santolla* due the increasing energy losses and the restriction in the limited stored energy in the form of yolk remaining

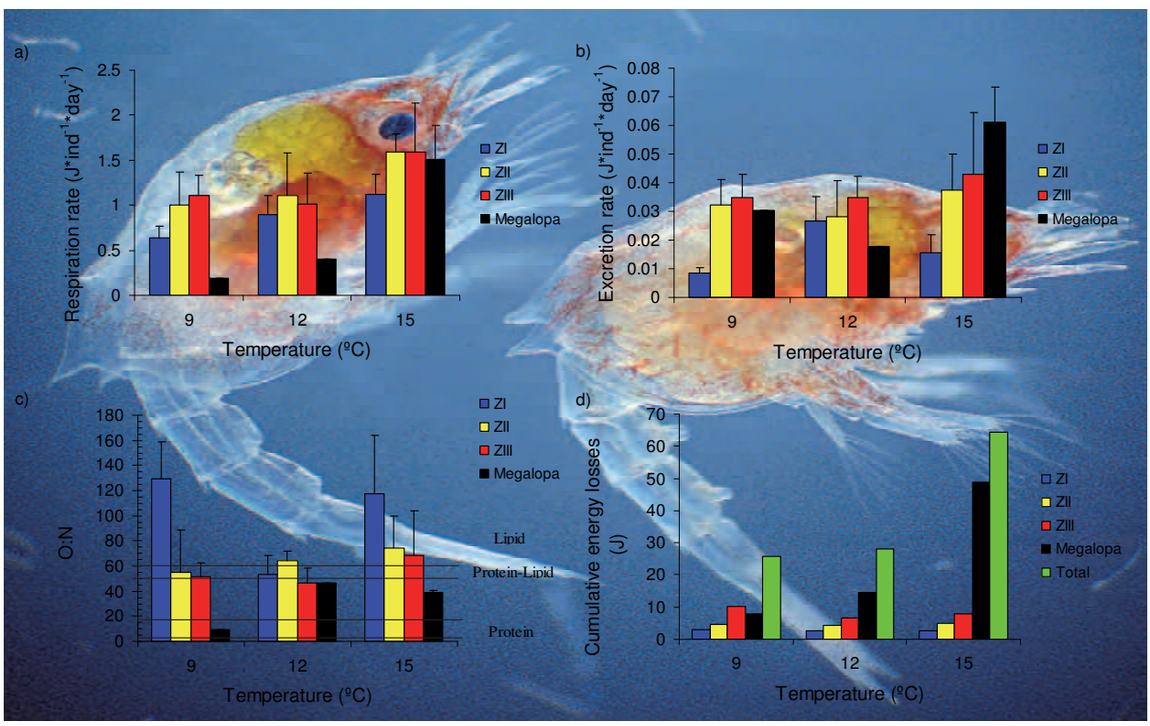


Fig. 7. Temperature effect on a) the respiration rate; b) the excretion rate (J*ind-1*day-1); c) O:N ratio; d) the cumulative energy losses (J) during larval development of *Lithodes santolla*.

from the embryonic development. For cultivation purposes physiological data suggest rearing temperatures below 15°C and special caution to reducing larval stress and wasteful energy losses.

After the increase in the atmospheric oxygen availability 2000 million of years ago, most animals were selected for a more efficient energy acquiring mechanism using oxygen as the last electron acceptor. While anaerobic pathways persist, aerobic ATP yield increase as much as 15 to 18-folds (depending on the metabolic pathway) the efficiency in the process of gaining energy Wieser, (1986). Hence, oxygen has a main role in obtaining energy for the cells, therefore for the organism. Oxygen decrease (hypoxia, both environmental and cellular; hypoxic generally refers to low oxygen conditions that are physiologically stressful, Levin, (2003)) has many effects in different levels, from population to individual and cellular, affecting the capability to obtain energy, which affects also the organism's activity and their growth performances. One of the most important mechanisms to deal with hypoxia is to improve the use of energy available, by means of reducing the metabolic rate. In fact, organisms consider well adapted to hypoxia events show a lower ATP consumption, to balance the reduction in the capability to generate ATP. During hypoxia, the ion pump slightly decreases, but the most important is the low in protein synthesis and degradation Hochachka & Somero, (2002). The reduction in protein synthesis result in an efficient energy saving mechanism and, at the same time, an effective mechanism to face hypoxic events, but for aquaculture purposes, such mechanisms activated by low dissolved oxygen (DO) results in a drop in the ingestion rate, animal activity and finally a marked reduction or even ceasing in growth. In marine animals, adaptation to low oxygen availability includes also modifying the acid-base balance in the hemolymph Martinez et al., (1998), hemocyanin binding capacity, oxyhemocyanin protein relationship, hemolymph osmolality, and ion concentrations as well as hemocyanin synthesis Johnson & Uglow, (1985); Charmantier et al., 1994; Chen & Kou, (1998); Taylor & Anstiss, (1999) Environmental parameters like high pH and low dissolved oxygen (DO) have been reported to cause reduction in hemocyte counts Cheng & Chen, (2000).

4.4 Dissolved oxygen as key factor in the bioenergetics of southern king crab juveniles

Crustacean aquaculture is widespread in tropical areas where higher water temperatures reduces oxygen solubility and accelerates the decomposition of organic material by bacteria and other oxygen consuming microorganisms both conducting to a decreasing in DO levels Rosas et al., (1998, 1999). For cold water organisms the relationship between DO and temperature described by Pörtner (2005) by the Metabolic Cold Adaptation hypothesis implies that the thermal tolerance of the animal is affected by the supply of oxygen to their mitochondria.

In an attempt to establishing the impact of culture environmental factors on the physiology of southern king crab *Lithodes santolla* with a final goal to develop cultivation techniques, the effect of dissolved oxygen (DO) on different physiological responses of *L. santolla* juveniles is analysed. Paschke et al. (2010) showed physiological responses in juveniles of *L. santolla* exposed 10 days to reduced Oxygen availability treatments (Fig. 8).

The juveniles regulate their metabolism until 4-9 kPa (ca. 4-6 mg $O2*l^{-1}$), maintaining their oxygen consumption rates, hemolymphatic protein levels, as well as the oxyhemocyanin

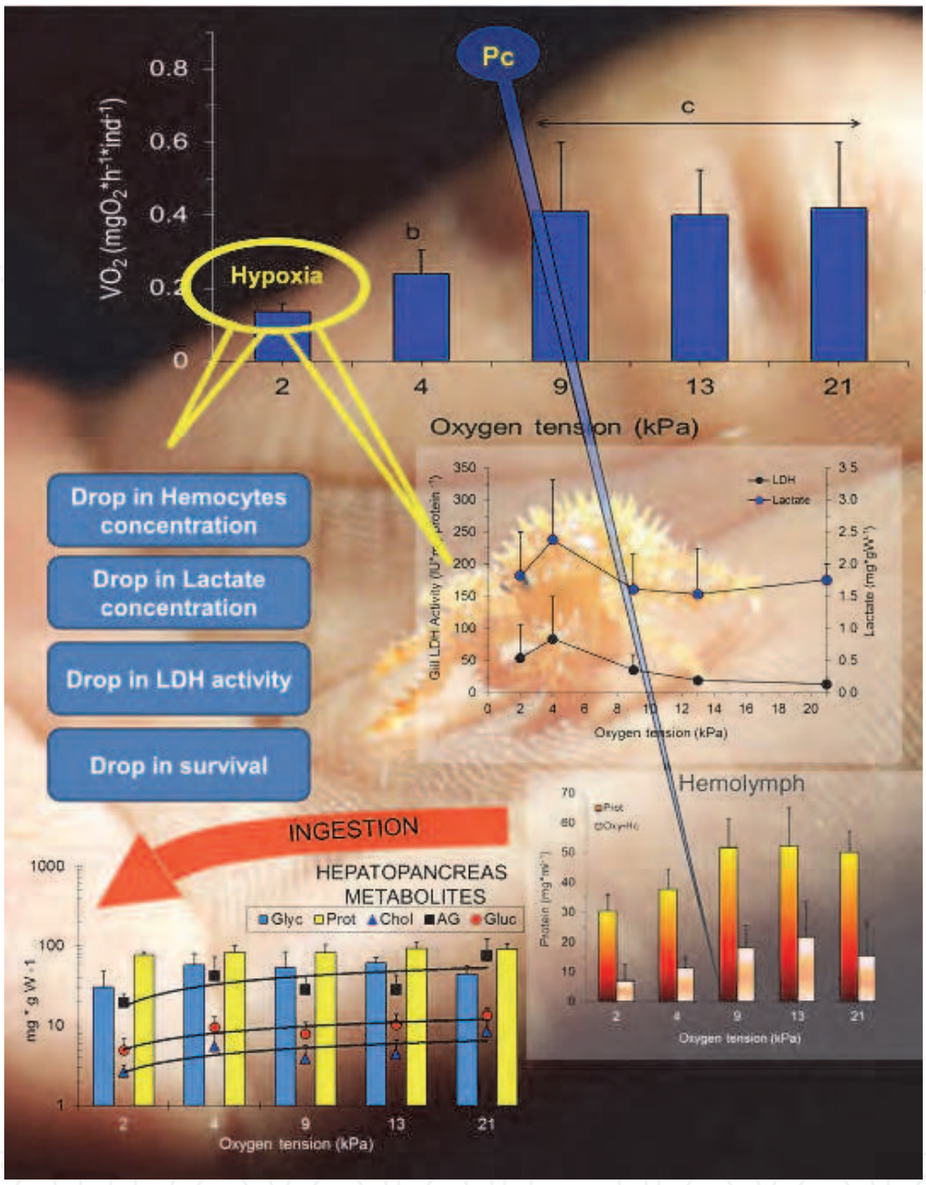


Fig. 8. Effect of dissolved oxygen (DO) on different physiological responses of *L. santolla* juveniles. Pc: critical point of aerobic metabolism shift; LDH: Lactate dehydrogenase; Prot: protein concentration; Oxy-Hc: oxygenated hemocyanin; Glyc: glycogen; Chol: cholesterol; AG: acylglycerids; Gluc: glucose.

concentration. These responses to reduced DO were complemented with anaerobic metabolism evidenced by the increase in the activity of gill-LDH and a consequent increase of Lactate concentration. A reduction in nutritional compounds in the hepatopancreas was interpreted as a consequence of a notorious reduction of food ingestion, and as an additional consequence of hypoxic conditions (the ingestion was affected severely and showed a linear relationship to dissolved oxygen (DO) between 2 and 13 kPa: 0.59 and 1.92 mg Dry weight h-1 ind-1, respectively). Severe chronic hypoxia (1 mgO2*l-1 or 2 kPa for 10 days) led to an unsustainable reduction in the aerobic and anaerobic activity, where a reduction of the gill-

LDH activity and Lactate concentration as well as oxygen consumption coincide with a significant reduction in hemolymphatic hemocytes and finally a survival reduction to 70%.

In accordance with these results and applying Ecophysiology to the development of cultivation techniques, the rearing systems were modified to ensure the adequate Oxygen supply in the first millimeter over the bottom surface, the place where juveniles of the southern king crab *Lithodes santolla* feed, moult and growth.

5. Modelling individual growth: Size-at-age variability in cephalopods and mixed models tools

Both individual and population growth is central to aquaculture practice and research, and modelling growth has been an important aim in methodological and theoretical studies Moltschaniwskyj, (2004); Vigliola & Meekan, (2009). Models have been extensively developed and applied to understand population growth (e.g. Malthussian models, logistic models, Leslie matrix, etc.), and those for individual growth have followed closely (von Bertalanffy model). Methods for measuring and modelling individual growth can be classified as either indirect or direct Semmens et al.,(2004). Indirect methods are those using modal progression analysis based on length-frequency data obtained from animals caught in the wild. These have been used to predict length-at-age in individuals of a variety of both vertebrate and invertebrate fisheries Solis-Ramírez, (1997); Cortez et al., (1999) Direct methods, such as mark and recapture using a variety of tags, have been used to follow the growth of juvenile and adult stages in the wild Sousa Reis & Fernándes, (2002); Cortez et al., (1999).

From an ecophysiological point of view, however, more useful are direct methods examining the growth of individuals whose age and specific culture conditions are known. The growth of an individual is the result of a series of energy transformations that begin with food ingested, together with the balance between the uses and destinations of the energy contained in that food Lucas, (1993); Rosas et al., (2007). Thus, the growth curve represents the manner in which the physiological and energy demands are expressed over time O'Dor & Wellls, (1987); Pauly, (1998) at differing levels of biological organization, i.e. body size, organs, tissue, and cells Moltschaniwskyj, (2004). In the context of energy balance, the ultimate need to measure and compare the way in which individuals grow becomes clear: individual growth models can be used not only to predict size at age, hence biomass productions at certain points in time, but can also serve to estimate growth curve parameters that can later be compared amongst different culture conditions.

The mathematical tool generally used to obtain growth curve parameters is least square fitting, which can be used both for linear and non-linear models. The linear least-squares problem occurs in statistical regression analysis where it has a closed-form solution. The non-linear problem has no closed-form solution and is usually solved by iterative refinement, where chi-square (X^2) and probability (p) values are obtained and evaluated at each iteration.

Analyses of individual growth have commonly involved fitting linear regression models of weight against time. However, such an approach involves difficulties because the weight vs. time relationship is rarely linear and, when it is, it is only for very short and specific periods. Moreover, repeated measurement of the same individuals violates an indispensible

requirement of regression models, namely that each data point be independent from others Zar, (1999); Zuur et al., (2007). Finally, size-at-age variation generally increases with age, resulting in strong heterogeneity of variance in different ages, another impediment for ordinary regression analysis Zuur et al., (2007). These characteristics violate important regression assumptions, thus producing unreliable models, i.e. models with dubious F, X^2 and p values that cannot be used for prediction purposes.

A methodological approach is fitting a regression model to the weight of each individual at a known fixed time (t_2) against its weight at an earlier point in time (t_1). This method ensures linearity of the X–Y function and independence of data points (as long as they are individually labelled). The resulting linear equation describes a type of relative growth, and its slope represents the proportionality of the difference between two individuals at t_2 relative to the difference between them at t_1. In turn, the line's intercept represents the final weight reached by the smallest individual in the dataset. In this context, comparing the slopes of different lines gives information on how much inter-individual weight differences change over experimental time. Concomitantly, comparing different line intercepts informs on individual growth rates: lines with different intercepts indicate different growth rates, because two animals with the same initial weight reach different final weights within the same period. Although this approach allows indirect corroboration of whether individuals with different final size have different growth rates, it does not permit estimation (mean value ± standard error) of those parameters in the equation that describe individual growth over time.

Generalized linear mixed models (GLMM) are a statistical tool that can complement growth analyses, because they allow modelling of the large variability in individual size observed within a culture population. GLMM can be applied to non-normal data in which random effects are present (Zuur et al., 2009). By incorporating components that modify the structure of variance, mixed models yield more-reliable estimators of model coefficients. In addition, through certain variance and correlation structures, mixed models may produce new parameters that estimate size-at-age variability and the time elapsed for two size measures to be statistically unrelated. GLMM procedures include the validation of models by visual inspection of residuals Montgomery & Peck, (1992); Draper & Smith, (1998.), thereby assuring that regression assumptions are adequately met.

Cephalopod growth has some remarkable characteristics: (i) growth rates are among the highest in metazoans (the highest in invertebrate metazoans, higher than those of fish and similar to those of mammals) Calow, (1987); (ii) it lacks an asymptotic growth phase Moltschaniwskyj, (2004); (iii) it is highly plastic owing to its strong dependence on abiotic and biotic factors, mainly temperature Pecl et al., (2004), the amount and quality of food André et al., (2008), and sexual maturation Semmens et al., (2004); (iv) it follows a biphasic pattern (as it often does in captivity), consisting of an initial rapid exponential phase followed by a second phase, where growth slows down progressively (André et al., 2009); and (v) it is highly variable intra-specifically Pecl et al., (2004); Leporati et al., (2007).

Size-at-age variability has been attributed to the lack of a strong association between age and size of these soft bodied invertebrates, and is well documented both in wild and in culture populations Leporati et al., (2007); Leporati et al., (2008). This great variability, patent even under controlled temperature and food conditions, has been associated with

initial size Leporati et al., (2007), which in turn is highly variable both within and between broods. High coefficients of variation (>20%) in initial weight have been observed in several octopod species (*Octopus bimaculoides*, Forsythe & Van Heukelem, (1987); *Octopus diguetti*, DeRusha et al., (1989); *Octopus ocellatus*, Segawa & Nomoto, (2002). Intra-brood variation, however, could not be related to either the mother´s weight or day of hatching Briceño-Jacques et al., (2010). Results suggest that growth rate does not depend on initial size in *O. maya* Briceño et al., (2010), but small differences in size during early life can be amplified and accumulated in time Vigliola & Meekan, (2009). This means that inter-individual variation in initial size has to be considered in experimental designs and data analyses aimed at understanding cephalopod growth.

Octopus maya is endemic to the Yucatan Peninsula Voss & Solis Ramirez, (1966), and its culture has received considerable attention Martinez et al., (2011); Uriarte et al., (2011). This species provides an interesting biological model to test hypotheses on heterogeneity in growth amongst cultured siblings, and serves to illustrate problems that arise in growth analysis and possible solutions to them.

Juveniles of *O. maya* from a single female were used. The eggs were held at 28°C (± 1°C) in an artificial incubator (without maternal care) until hatching. The weight of a total of 84 juveniles, that hatched over the course of 8 d, was recorded 24 h after hatching ($W_1 \pm 0.01$g), and then again on days 15, 45, 75 and 105. Octopuses were housed individually in 300 ml containers connected to a recirculation system in which water temperature was kept constant at 27 ± 1 °C. Octopuses were fed live adult brine shrimp (*Artemia salina*) and pieces of blue crab (*Callinectes sapidus*) meat *ad libitum*. Overall mortality (at day 105) was 60.7%.

To obtain a curve of weight gain as a function of time, a linear model was adjusted to the relationship between the natural logarithm of W_1, W_{15}, W_{45}, W_{75}, and W_{105} weights of all individuals and their corresponding ages:

$$\mathbf{Y_{ij} = \alpha + \beta_1 X_i + e_{ij}}$$

Previous regression analysis, graphic representations of the data were explored to (i) identify extreme points (point graphs); (ii) assess normality (histograms and percentile graphs); and (iii) verify linear relationships *X*–*Y* graphs; Zuur et al., (2007). The regression was fitted with a generalized least square (GLS) procedure through restricted maximum likelihood (REML) and incorporated correlation and variance structures, using GLMM to ensure that homocedasticity and independence requisites were met (Fig. 9). Models featuring optimal correlation and variance structures were selected by considering values of the Akaike information criterion (AIC) and hypothesis tests based on *F* and likelihood ratio (L ratio) values. Once the significance of regression parameters were established through *F*, L ratio and *t*-tests, the model was validated by visual inspection of residuals Montgomery & Peck, (1992). We used the parameter δ as an estimator of the tendency of weight variances to increase with age.

Regression parameters differed significantly from 0 (Table 5). Interdependence of data over time (because we weighed animals repeatedly) resulted in a cyclic residual pattern, so we incorporated an autocorrelation structure (spherical spatial structure) in the random-effects term of the model (ε_i). Following Pinheiro & Bates, (2000), we kept this structure in the model, because (i) AIC values indicated that using it improved the model (AIC = 319.45),

and (ii) it made the cyclic residual pattern disappear. The estimated range parameter associated with this correlation structure was 74.3 (Table 5), representing the interval (in days) necessary to avoid correlation between two consecutive weight measurements of the same animal. Heterogeneity of variances was identified based on an increase in weight variation with age and was accounted for by including a variance structure of the type:

$$\varepsilon_i \sim N(0, \sigma^2 \bullet |age_i|^{2\delta})$$

Including this term significantly improved the model (L-ratio = 70.00; $p < 0.001$; AIC = 251.4). Finally, visual inspection of residuals revealed uniform dispersion and no evident patterns, and σ (a constant representing the increase in residual weight variation with age) was estimated to be 0.20 (Table 5).

Parameter	Value	Significance test
α	-2.182 ± 0.22 [a]	t = -100.92 [b]
β	0.030 ± 0.001	t = 48.60 [b]
σ	0.209	
δ	0.204	L.ratio = 70.00 [b]
Range	74.3	L.ratio = 114.20 [b]
AIC	251.4	

Table 5. Parameters of the exponential growth model: α = intercept; β = slope; σ = residual standard error; δ = variance structure parameter; range: correlation structure parameter defined by the variogram. Significance values of statistics used are also shown; [a] log-transformed values; [b] $p < 0.001$.

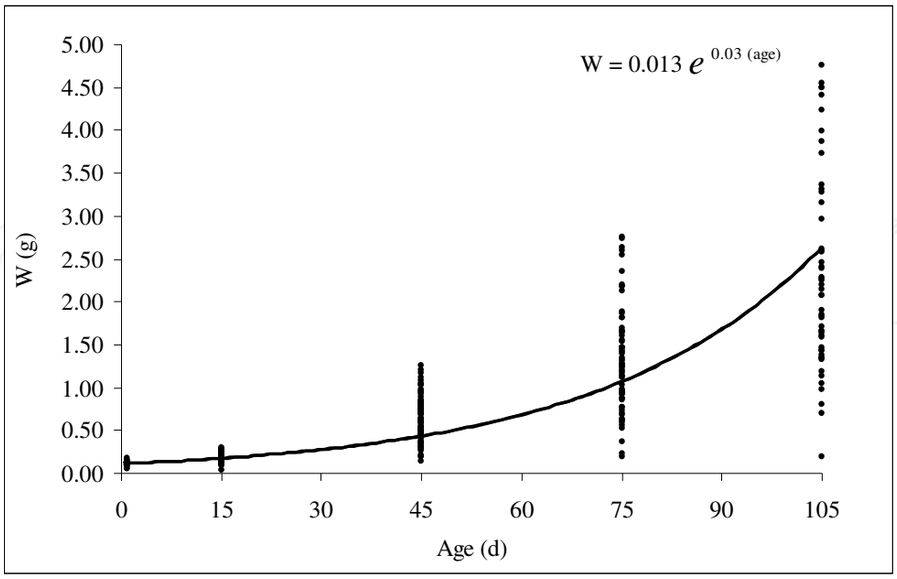


Fig. 9. Exponential relationship of weight and age (to age 105 d) in individually housed juvenile *Octopus maya* kept at 27 ± 1°C. Data points and continuous line are observed and predicted values, respectively.

The GLMM model included the use of a variance and a correlation structure that enhanced the model fit (the best AIC value was obtained: 251.4), and successfully explained octopus weight as a function of age. Because the date of hatching was registered for each individual, "age" is fixed in the model (i.e. it has no associated error), a feature in models fitted by least squares that is not usually met. According to this model, *O. maya* juveniles grow at a rate of 0.03 g/day, a rate similar to that obtained for other *O. maya* cultures (unpublished data). The δ parameter estimated in 0.204 is the exponent Given the correlation and variance structures included in the current model, and the parameters associated with them (δ, range), the procedures followed to adjust the GLMM model detailed here allow a high level of precision in predicting octopus weight from known age, thus, making the estimates of growth rate more reliable than other growth models.

6. Conclusions

During aquaculture technological development of species it is common that farmers or researchers start the culture only putting some animals in ponds, floating cages, or any other closed environment giving attention on productive parameters such as growth and survival. It is also common that, at the beginning of culture, animals are feed with the local available food in attempt to evaluate if in such conditions animals can be raised. However, this approach does not allow getting enough information for a sustainable development of culturing technology. In this sense, ecophysiology can help to evaluate how environmental conditions modulate several responses of cultured organisms, including the scope for growth, one of the key parameters involved in the energy balance equation. This model could be used as a base to make economical inferences or to modify culture methods. As showed in the present work, independently of the cultured species, the bulk of papers dedicated to demonstrate the effects of many environmental factors on physiological condition of animals validate applied ecophysiology as a useful method for the development and improvement of cultivation technology of aquatic organisms.

7. Acknowledgements

The present chapter is the result of many research made with the financial support of FONDEF D02i1163, D05i10217, Fondecyt 1110637, FONDEF D09I1153 in Chile and CONACYT through their financial support to make the sabbatical year of CR and Papiit IN 290327 to CR in Mexico.

8. References

Adachi, K., Hirata, T., Nishioka, T., Sakaguchi, M. (2003). Hemocyte components in crustaceans convert hemocyanin into a phenoloxidase-like enzyme. *Comparative Biochemical and Physiol*ogy, 134B, 135-141.

Anderson, R.C., Wood, J.B. (2001). Enrichment for giagiant Pacific octopuses: happy as a clam? *Journal Applied Animal Welfare Science,* 4, 157-168.

André, J., Grist, E.P.M., Semmens, J.M., Pecl, G., Segawa, S. (2009). Effects of temperature on energetics and the growth pattern of benthic octopuses. *Journal of Experimental Marine Biology and Ecology*, 374, 167-179.

André, J., Pecl, G., Semmens, J.M., Grist, E.P.M. (2008). Early life-history processes in benthic octopus: Relationships between temperature, feeding, food conversion, and growth in juvenile *Octopus pallidus*. *Journal of Experimental Marine Biology and Ecology*, 354, 81-92.

Anger, K., Lovrich, G., Thatje, S., Calcagno, J. (2004). Larval and early juvenile development of *Lithodes santolla* (Molina, 1782) (Decapoda: Anomura: Lithodidae) reared at different temperatures in the laboratory. *Journal of Experimental Marine Biology and Ecology*, 306, 217-230.

Arena, L., Cuzon, G., Pascual, C., Gaxiola, G., Soyez, C., VanWormhoudt, A., Rosas, C. (2003). Physiological and genetic genetic variations in domesticated and wild populations of *Litopenaeus vannamei* fed with different carbohydrtaes levels. *Journal of Shellfish Research,* 22, 1-11.

Boletzky, S.V. (2003). Biology of early life in cephalopod molluscs. *Advances in Marine Biology*, 44, 144-203.

Boyd, C.E., (1995). Soil and water quality management in aquaculture ponds. INFOFISH International. Kuala Lumpur [INFOFISH INT.], 29-32.

Bradley, E.A. (1974). Some observations of *Octopus joubini* reared in an inland aquarium. *Journal of Zoology London*, 173, 355-368.

Bray, W.A., Lawrence, A.L., Leung-Trujillo, J.R. (1994). The effect of salinity on growth and survival of *Penaeus vannamei*, with observations on the interaction of IHHN virus and salinity. *Aquaculture*, 122, 133-146.

Briceño-Jacques, F., Mascaró, M., Rosas, C. (2010). GLMM-based modelling of growth in juvenile *Octopus maya* siblings: does growth depend on initial size? *ICES Journal of Marine Science*, 67, 1509-1516.

Brito, R., Chimal, Rosas, C. (2000). Effect of salinity in survival, growth and osmitic capacity of early juveniles of Farfantepenaeus brasiliensis (decapoda; penaeidae). *Journal of Experimental Marine Biology and Ecology* 244, 253-263.

Calow, P., (1987). Evolutionary physiological ecology? In: *Evolutionary physiological ecology*, Calow, P. (Ed.),. Cambridge University Press, New York, pp. 1-7.

Calcagno, J.A., Anger, K., Lovrich, G.A., Thatje, S., Kaffenberger, A. (2004). Larval development of the subantarctic king crabs *Lithodes santolla* and *Paralomis granulosa* reared in the laboratory. *Helgoland Marine Research*, 58, 11-14.

Charmantier, G., Soyez, C. and Aquacop (1994). Effect of moult stage and hypoxia on osmoregulatory capacity in the peneid shrimp *Penaeus vannamei*. *Journal of Experimental Marine Biology and Ecology* 178, 223–246

Chen, J.C. and Kou, T.T. (1998) Hemolymph acid–base balance, oxyhemocyanin, and protein levels of *Macrobrachium rosenbergii* at different concentrations of dissolved oxygen. *Journal of Crustacean Biology,* 18, 437–441

Cheng, W., Chen, J.C. (2000). Effects of pH, temperature and salinity on immune parameters of the freshwater prawn *Macrobrachium rosenbergii*. *Fish and Shellfish Immunology* 10,387–391

Claybrook, D.L. (1983). Nitrogen metabolism. In: *The Biology of Crustacea* L.H, M. (Ed.), Internal anatomy and physiological regulation. Academic Press, New York, pp. 163-213.

Cortez, T., Gonzalez, A.F., Guerra, A. (1999). Growth of cultured *Octopus mimus* (Cephalopoda: Octopodidae). *Fisheries Research*, 40, 81-89.

Chen, J.C., Lin, M.N., Lin, J.Y., Ting, Y.Y. (1992). Effect of salinity on growth of *Penaeus chinensis* juveniles. *Comparative Biochemical and Physiology*, 102A, 343-346.

Danbom, D.B. (1979.) *The resisted revolution: urban America and the industrialization of agriculture, 1900 - 1930*. Iowa State University Press, Ames.

Danford, A., Uglow, R.F., Rosas, C., .Physiological responses of blue crabs (*Callinectes* sp) to procedures used in the soft crab fishery in La Laguna de T,rminos, México. In: B.C. Paust and A.A. Rice (eds). Marketing and Shipping Live Aquatic Products: Proceedings of the Second International Conference and Exhibition, November 1999, Seatle Washington, University of Alaska Sea Grant, AK-SG-01-03, Fairbanks: 1-8

Decamp, O., Cody, J., Conquest, L., Delanoy, G., Tacon, A. (2003). Effect of salinity on natural community and production of *Litopenaeus vannamei* (Boone), within experimental zero-water exchange culture systems. *Aquaculture Research,* 34, 345-355.

DeRusha, R.H., Forsythe, J.W., DiMarco, F.P., Hanlon, R.T. (1989). Alternative diets for maintaining and rearing cephalopods in captivity. *Laboratory Animal Science*, 39, 306-312.

Destoumieux, D., Mo¤oz, M., Bulet, P., Bachere, E. (2000). Peneidins, a family of anitimicrobial peptides from penaeid shrimp (Crustace, Decapoda). *Cellular and Molecular Life Sciences*, 57, 1260-1271.

Destoumieux, D., Saulnier, D., Garnier, J., Jouffrey, C., Bulet, P., Bachere, E. (2001). Antifungal peptides are generated from the C terminus of shrimp hemocyanin in response to microbial challenge. *The Journal of Biological Chemistry*, 276, 47070-47077.

Draper, N., Smith, H. (1998). *Applied Regression Analysis*. John Wiley, New York.

FAO (2011). *El estado mundial de la pesca y la acuicultura 2010*. Organización de las Naciones Unidas para la agricultura y alimentación. Departamento de Pesca y Acuicultura de la FAO.

Farías, A., García-Esquivel, Z., Viana, M.T. (2003). Physiological energetics of green abalone *Haliotis fulgens*, fed on a balanced diet. *Journal of Experimental Marine Biology and Ecology*, 289, 263-276.

Farias, A., Uriarte, I. (2006). Nutrition in pectinids. In: Scallops: *Biology, ecology and aquaculture*, Shumway, S.E., Parsons, G.J. (Eds.). Elsevier, pp. 521-542.

Farías, A., Uriarte, I. (2001). Effect of microalgae protein on the gonad development and physiological parameters for the scallop *Argopecten purpuratus* (Lamarck, 1819). *Journal of Shellfish Research,* 20, 97-105.

Farías, A., Uriarte, I., Castilla, J.C. (1998). A biochemical study of the larval and postlarval satges of the Chilean scallop *Argopecten purpuratus*. *Aquaculture,* 166, 37-47.

Farias, A., Uriarte, I., Hernández, J., Pino, S., Pascual, C., Caamal, C., Domingues, P., Rosas, C., 2009. How size relates to oxygen consumption, ammonia excretion, and ingestion rates in cold (*Enteroctopus megalocyathus*) and tropical (*Octopus maya*) octopus species. *Marine Biology*, 156, 1547-1558.

Farrell, A.P. (Ed). (2011). *Enciclopedia of fish physiology: from genome to environment*. Academic Press, New York.

Ferreiro, M.J., Pérez-Camacho, A., Labarta, U., Beiras, R., Planas, M.D., Férnandez-Reiriz, M.J. (1990). Changes in the biochemical composition of *Ostrea edulis* larvae fed on different food regimes. *Marine Biology*, 106, 395-401.

Flores, E.E.C. (1983). Visual discrimination testing in the squid *Tadores pacificus*: experimental evidence for lack of color vision. *Memoires of the National Museum Victoria*, 44, 213-227.

Forsythe, J.W., Van Heukelem, W.F. (1987). Growth. In: *Cephalopod life cycles*, Boyle, P.R. (Ed.),. Academic Press, London, pp. 135-155.

García-Garrido, S., Domingues, P., Navarro, J.C., Hachero-Cruzado, I., Garrido, D., Rosas, C. (2011). Growth, partial energy balance, mantle and digestive gland lipid composition of *Octopus vulgaris* (Cuvier, 1797) fed with two artificial diets. *Aquaculture Nutrition* 17, e174-e187.

Hall, M., Wang, R., Van Antwerpen, R., Sottrup-Jensen, L., S"derhall, K. (1999). The crayfish plasma clotting protein: a vitellogenin-related protein responsible for clot formation in crustacean blood. *Proceedings of the National Academy of Sciences USA,* 96, 1965-1970.

Hanlon, R.T., Turk, P.E., Lee, P.G. (1991). Squid and cuttlefish mariculture: An update perspective. *Journal of Cephalopod Biology*, 2, 31-40.

Hanlon, R.T., Wolterding, M.R. (1989). Behavior, body patterning, growth and life history of *Octopus briareus* cultured in the laboratory. *American Malacological Bulletin,* 7, 21-45.

Hernández, J., Uriarte, I., Viana, M.T., Westermeier, R., Farias, A. (2009). Growth performance of weaning red abalone (*Haliotis rufescens)* fed with *Macrocystis pyrifera* plantlets and *Porphyra columbina* compared with a formulated diet. *Aquaculture Research,* 40, 1694-1702.

Hochachka, P.W. and Somero, G.N. (2002) *Biochemical adaptation: mechanism and process in physiological evolution*. New York: Oxford University Press

Hochner, B., Shomrat, T., Fiorito, G. (2006). The octopus: a model for a comparative analysis of evolution of learning and memory mechanisms. *Biological Bulletin*, 210, 308-317.

Johnson, I. and Uglow, R.F. (1985) Some effects of aerial exposure respiratory physiology and blood chemistry of *Carcinus maenas* (L) and *Liocarcinus puber* (L). *Journal of Experimental Marine Biology and Ecology*, 94,1–12

Kattner, G., Graeve, M., Calcagno, J.A., Lovrich, G.A., Thatje, S., Anger, K. (2003). Lipid, fatty acid and protein utilization during lecithotrophic larval development of *Lithodes santolla* (Molina) and *Paralomis granulosa* (Jacquinot). *Journal of Experimental Marine Biology and Ecology,* 292, 61-74.

Iglesias, J., Sanchez, F.J., Bersano, J.F.G., Carrasco, J.F., Dhont, J., Fuentes, L., Linares, F., Muñoz, J.L., Okumura, K., Roo, J., van der Meeren, T., Vidal, E., Villanueva, R. (2007). Rearing of *Octopus vulgaris* paralarvae: Present status, bottlenecks and trends. *Aquaculture*, 266, 1-15.

Katsanevakis, S., Stephanopoulou, S., Miliou, H., Moraitou-Apostolopoulou, M., Verriopoulos, G. (2005). Oxygen consumption and ammonia excretion of *Octopus vulgaris* (Cephalopoda) in relation to body mass and temperature. *Marine Biology,* 146, 725-732.

Leporati, S., Pecl, G.T., Semmens, J.M. (2007). Cephalopod hatchling growth: the effects of initial size and seasonal temperatures. *Marine Biology*, 151, 1375-1383.

Leporati, S.C., Pecl, G.T., Semmens, J.M. (2008). Reproductive status of *Octopus pallidus*, and its relationship to age and size. *Marine Biology*, 155, 375-385.

Levin, L.A. (2003) Oxygen minimum zone benthos: adaptation and community response to hypoxia. *Oceanography and Marine Biology: An Annual Review*, 41,1– 45

Lovatelli, A., Farías, A., Uriarte, I. (Eds.) (2007). *Estado actual del cultivo y manejo de mosluscos bivalvos y su proyección futura: factores que afectan su sustentabilidad en América Latina*. Taller técnico de la FAO, 20-24 de agosto 2007, Puerto Montt, Chile. FAO, Roma, Italy.

Lovrich, G.A., Thatje, S., Calcagno, J.A., Anger, K., Kaffenberger, A. (2003). Changes in biomass and chemical composition during lecithotrophic larval development of the southern king crab, *Lithodes santolla* (Molina). *Journal of Experimental Marine Biology and Ecology*, 288, 65- 79.

Lucas, A. (1993). *Bioénergétique Des Animaux Aquatiques*. Masson, Paris.

Mair, J.M. (1980). Salinity and water type preferences of four species of postlarval shrimp (Penaeus) from Wets México. *Journal of Experimental Marine Biology and Ecology*, 45, 69-82.

Martinez, E., Aguilar, M., Trejo, L., Hernández, I., Díaz-Iglesia, E., Soto, L.A., Sanchez, A. and Rosas, C, (1998). Lethal low oxygen disolved oxygen concentrations for postlarvae and early juvenile *Penaeus setiferus* at different salinities and pH. *Journal of the World Aquaculture Society,* 29(2):221–229

Martinez, R., Lopez-Ripoll, E., Avila-Poveda, O., Santos-Ricalde, R., Mascaro, M., Rosas, C. (2011). Cytological ontogeny of the digestive gland in post-hatching *Octopus maya* , and cytological background of digestion in juveniles. *Aquatic Biology,* 11, 249-261.

Mayzaud, P. and Conover, R. (1988). O:N atomic ratio as a tool to describe zooplankton metabolism. *Marine Ecology Progress Series,* 45, 289-302.

McFarland, W.N., Lee, B.D. (1963). Osmotic and ionic concentrations of penaeid shrimps of Texas. *Bulletin of Marine Science of the Gulf and Caribbean* ,13, 391-417.

McGraw, W.J., Davies, D.A., Teichert-Coddington, D.R., Rouse, D.B. (2002). Acclimation of *Litopenaeus vannamei* postlarvae to low salinity: Influence of age, salinity endopoint and rate of salinity reduction. *Journal of the World Aquaculture Society* 33, 78-84.

Miliou, H., Fintikaki, M., Kountouris, T., Verriopoulos, G. (2005). Combined effects of temperature and body weight on growth and protein utilization of the common octopus *Octopus vulgaris*. *Aquaculture,* 249, 245-256.

Moltschaniwskyj, N.A. (2004). Understanding the process of growth in cephalopods. *Marine and Freshwater Research,* 55, 379–386.

Montaño-Pérez, K., Yepiz-Plascencia, G., Higuera-Ciapara, I., Vargas-Albores, F. (1999). Purification and characterization of the clotting protein from the white shrimp *Penaeus vannamei. Comparative Biochemical and Physiology*, 122, 381-387.

Montgomery, D.C., Peck, E.A. (1992). *Introduction to Linear Regression Analysis*. John Wiley, New York.

O'Dor, R.K., Wellls, M.J. (1987). Energy and nutrient flow. In: *Cephalopod life cycles*, O'Dor, R.K., Wellls, M.J. (Eds.),. Academic Press, London, pp. 109-131.

Ogle, J.T., Beaugez, K., Lotz, J.M. (1992). Effects of salinity on survival and growth of postlarval *Peaneus vannamei*. *Gulf Research Reports,* 8, 415-421.

Palacios, E., Bonilla, A., Perez, A., Racotta, I.S., Civera, R. (2004). Influence of highly unsaturated fatty acids on the responses of white shrimp (*Litopenaeus vannamei*) postlarvae to low salinity. *Aquaculture*, 299 201-215.

Paschke, K., Cumillaf, J.P., Loyola, S., Gebauer, P., Urbina, M., Chimal, M.E., Pascual, C. and Rosas, C. (2010). Effect of dissolved oxygen level on respiratory metabolism, nutritional physiology, and immune condition of southern king crab *Lithodes santolla* (Molina, 1782) (Decapoda, Lithodidae). *Marine Biology,* 157:7-18.

Pascual, C., Gaxiola, G., Rosas, C. (2003). Blood metabolites and hemocyanin of the white shrimp *Litopenaeus vannamei* : the effect of culture conditions and a comparison with other crustacean species. *Marine Biology,* 142, 735-745.

Pascual, C., Arena, L., Cuzon, G., Gaxiola, G., Taboada, G., Valenzuela, M., Rosas, C. (2004a). Effect of a size-based selection program on blood metabolites and immune response of *Litopenaeus vannamei* juveniles fed different dietary carbohydrate levels. *Aquaculture*, 230, 405-416.

Pascual, C., Zenteno, E., Cuzon, G., Sanchez, A., Gaxiola, G., Taboada, G., Suarez, J., Maldonado, T., Rosas, C. (2004b). Litopenaeus vannamei juveniles energetic balance and immunological response to dietary protein. *Aquaculture*, 236 431-450

Pascual, C., Sanchez, A., Zenteno, E., Cuzon, G., Gaxiola, G., Brito, R., Gelabert, R., Hidalgo, E., Rosas, C. (2006). Biochemical, physiological, and immunological changes during starvation in juveniles of *Litopenaeus vannamei*. *Aquaculture*, 251, 416-429.

Pauly, D. (1998). Why squid, though not fish, may be better understood by pretending they are. *South African Journal of Marine Science*, 20, 47–58.

Pecl, G., Steer, M.A., Hodgson, K. (2004). The role of hatchling size in generating the intrinsic size-at-age variability of cephalopods: extending the *Forsythe hypothesis*. *Marine Freshwater Research*, 55.

Perazzolo, L.M., Barracco, M.A. (1997). The prophenoloxidase activating system of the shrimp *Penaeus paulensis* and associated factors. *Developmental and Comparative Immunology* , 21, 385-395.

Pinheiro, J.C., Bates, D.M. (2000). *Mixed-Effects Models in S and S-Plus*. Springer, New York.

Ponce-Palafox, J., Mert¡nez-Palacios, C.A., Ross, L.G. (1997). The effects of salinity and temperature on the growth and survival rates of juveniles white shrimp *Penaeus vannamei* , Boone, 1931. *Aquaculture,* 157, 107-115.

Pörtner, H.O., Storch, D., Heilmayer, O. (2005). Constraints and trade-offs in climate-dependent adaptation: energy budgets and growth in a latitudinal cline. *Scientia Marina,* 69 (Suppl. 2), 271-285

Rosas, C., Bolongaro-Crevenna, A., Sanchez, A., Gaxiola, G., Soto, L., Escobar, E. (1995). Role of the digestive gland in the energetic metabolism of *Penaeus setiferus. Biological Bulletin*, 189, 168-174.

Rosas, C., Martínez E., Gaxiola G., Brito R., Díaz-Iglesia E., Soto L.A. (1998). Effect of dissolved oxygen on the energy balance and survival of *Penaeus setiferus* juveniles. *Marine Ecology Progress Series,* 174, 67-75.

Rosas, C., Martinez, E., Gaxiola, G., Brito, R., Sanchez, A., Soto, L.A. (1999). The effect of dissolved oxygen and salinity on oxygen consumption, ammonia excretion and osmotic pressure of *Penaeus setiferus* (Linnaeus) juveniles. *Journal of Experimental Marine Biology and Ecology,* 234, 41-57.

Rosas, C., Cuzon, G., Gaxiola, G., Arena, L., Lemaire, P., Soyez, C., Van Wormhoudt, A. (2000). Influence of dietary carbohydrate on the metabolism of juvenile *Litopenaeus stylirostris. Journal of Experimental Marine Biology and Ecology,* 249, 181-198.

Rosas, C., Cuzon, G., Gaxiola, G., LePriol, Y., Pascual, C., Rossignyol, J., Contreras, F., Sanchez, A., Van Wormhoudt, A. (2001a). Metabolism and growth of juveniles of *Litopenaeus vannamei*: effect of salinity and dietary carbohydrate levels. *Journal of Experimental Marine Biology and Ecology,* 259 1-22.

Rosas, C., Cuzon, G., Taboada, G., Pascual, C., Gaxiola, G., Van Wormhoudt, A. (2001b). Effect of dietary protein and energy levels (P/E) on growth, oxygen consumption, hemolymph and digestive gland carbohydrates, nitrogen excretion and osmotic pressure of *Litopenaeus vannamei* and *L. setiferus* juveniles (Crustacea, Decapoda ; Penaeidae). *Aquaculture Research,* 32 1-20.

Rosas, C., Cuzon, G., Gaxiola, G., Pascual, C., Taboada, G., Arena, L., VanWormhoudt, A. (2002). An energetic and conceptual model of the physiological role of dietary carbohydrates and salinity on *Litopenaeus vannamei* juveniles. *Journal of Experimental Marine Biology and Ecology* 268, 47-67.

Rosas, C., Cuzon, G., Pascual, C., Gaxiola, G., Lopez, N., Maldonado, T., Domingues, P. (2007). Energy balance of *Octopus maya* fed crab and artificial diet. *Marine Biology,* 152, 371-378.

Scheel, D. (2002). Characteristics of habitats used by *Enteroctopus dofleini* in prince William Sound and Cook Inlet, Alaska. *Marine Ecology,* 23, 185-206.

Schmitt, A.S.C., Uglow, R.F. (1997). Hemolymph constituent levels and ammonia efflux rates of *Nephrops norvegicus* during emersion. *Marine Biology,* 127, 403-410.

Segawa, S., Nomoto, A. (2002). Laboratory growth, feeding, oxygen consumption and ammonia excretion of *Octopus ocellatus*. *Bulletin of Marine Science*, 71, 801-813.

Semmens, J.M., Pecl, G.T., Villanueva, R., Jouffre, D., Sobrino, I., Wood, J.B., Rigby, P.R. (2004). Understanding octopus growth: patterns, variability and physiology. *Marine and Freshwater Research* 55, 367–377.

Smith, C.D. (2003). Diet of *Octopus vulgaris* in False Bay, South Africa. *Marine Biology*, 143, 1127-1133.

Solis-Ramírez, M.J. (1997). The Octopus maya fishery of the Yucatán Peninsula. In: *The Fishery and Market Potential of Octopus in California,* Hochberg, L.,Ambrose, E. (Eds.), CMSC: pp. 1-10. Los Angeles, CA., pp. 1-10.

Sousa Reis, C., Fernándes, R. (2002). Growth observations on *Octopus vulgaris* Cuvier 1797 from the Portuguese waters: growth lines in the vestigial shell as possible tools for age determination. *Bulletin of Marine Science*, 71, 1099-1103.

Sritunyalucksana, K., Soderhall, K. (2000). The proPO and clotting system in crustaceans. *Aquaculture,* 191, 53-69.

Staples, D.J., Heales, D.S. (1991). Temperature and salinity optima for growth and survival of juveniles banana prawns *Penaeus merguiensis*. *Journal of Experimental Marine Biology and Ecology*, 154, 251-274.

Summer, W.C., McMahon, J.J. (1970). Survival of unfed squid, *Loligo pealei* in an aquarium. *Biological Bulletin*, 138, 389-396.

Sykes, A.V., Domingues, P., Correia, M., Andrade, P. (2006). Cuttlefish culture - state of art and future trends. *Vie et Millieu*, 56, 129-137.

Taylor, H.H. and Anstiss, J.M. (1999). Copper and haemocyanin dynamics in aquatic invertebrates. *Marine Freshwater Research*, 48,889–897

Thatje, S., Calcagno, J.A., Lovrich, G.A., Sartoris, F.J., Anger, K. (2003). Extended hatching periods in the subantarctic lithodid crabs *Lithodes santolla* and *Paralomis granulosa* (Crustacea: Decapoda: Lithodidae). *Helgoland Marine Research*, 57, 110-113.

Uriarte, I., Farías, A. (1999) The effect of dietary protein content of growth and biochemical composition of Chilean scallop *Argopecten purpuratus* (L.) postlarva and spat. *Aquaculture,* 180, 119-127.

Uriarte, I., Iglesias, J., Rosas, C., Viana, M.T., Navarro, J.C., Seixas, P., Vidal, E., Ausburger, A., Pereda, S., Godoy, F., Paschke, K., Farias, A., Olivares, A., Zuñiga, O. (2011). Current status and bottle neck of octopod aquaculture: the case of American species. *Journal of the World Aquaculture Society* In press.

Vargas-Albores, F., Yepiz-Plascencia, G. (2000). Beta glucan binding protein and its role in shrimp immune response. *Aquaculture* , 191, 13-21.

Vigliola, L., Meekan, M.G. (2009). The back-calculation of fish growth from otoliths. . In: *In Tropical Fish Otoliths: Information for Assessment, Management and Ecology*, Green, B.S., Mapstone, B.M., Carlos, G., Begg , G.A. (Eds.), Springer, New York, pp. 174–211.

Villanueva, R. (1993). Diet and mandibular growth of *Octopus magnificus* (Cephalopoda). *South African Journal of Marine Science* 13, 121-126.

Villanueva, R., Norman, M.D. (2008). Biology of the planktonic stages of benthic octopuses. *Oceanography and Marine Biology - Annual Review* 46, 105-202.

Voss, G.L., Solis Ramirez, M.J., 1966. *Octapus maya*, a new species from the Bay of Campeche, Mexico. *Bulletin Marine Science*, 16, 615-625.

Weiner, D.R. (2000). *Models of Nature: Ecology, Conservation and Cultural Revolution in Soviet Russia*. University of Pittsburg Press, USA.

Wieser,W. (1986). *Bioenergetik. Energietransformationen bei Organismen*. Georg-Thieme-Verlag, Stuttgart, 245 pp.

Wollesen, T., Loesel, R., Wanninger, A. (2009). Pygmy squids and giant brains: Maping the compley cephalopod CNS by phalloidin staining of vibratome sections and whole-mount preparations. *Journal of Neuroscience Methods*, 179, 63-67.

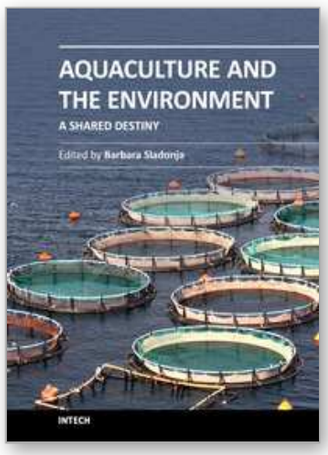
Zar, J.H. (1999). *Biostatistical Analysis*. Prentice-Hall, New Jersey.

Zuur, A.F., Ieno, E.N., Smith, G.M. (2007). *Analysing Ecological Data Series: Statistics for Biology and Health*. Springer, New York.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M. (2009). *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York.

IntechOpen

IntechOpen



Aquaculture and the Environment - A Shared Destiny

Edited by Dr. Barbara Sladonja

ISBN 978-953-307-749-9
Hard cover, 246 pages
Publisher InTech
Published online 22, December, 2011
Published in print edition December, 2011

Aquaculture is the art, science and business of cultivating aquatic animals and plants in fresh or marine waters. It is the extension of fishing, resulted from the fact that harvests of wild sources of fish and other aquatic species cannot keep up with the increased demand of a growing human population. Expansion of aquaculture can result with less care for the environment. The first pre-requisite to sustainable aquaculture is clean wate, but bad management of aquatic species production can alter or even destroy existing wild habitat, increase local pollution levels or negatively impact local species. Aquatic managers are aware of this and together with scientists are looking for modern and more effective solutions to many issues regarding fish farming. This book presents recent research results on the interaction between aquaculture and environment, and includes several case studies all over the world with the aim of improving and performing sustainable aquaculture.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Carlos Rosas, Cristina Pascual, Maite Mascaró, Paulina Gebauer, Ana Farias, Kurt Paschke and Iker Uriarte (2011). Applied Ecophysiology: An Integrative Form to Know How Culture Environment Modulates the Performance of Aquatic Species from an Energetic Point of View, Aquaculture and the Environment - A Shared Destiny, Dr. Barbara Sladonja (Ed.), ISBN: 978-953-307-749-9, InTech, Available from: http://www.intechopen.com/books/aquaculture-and-the-environment-a-shared-destiny/applied-ecophysiology-an-integrative-form-to-know-how-culture-environment-modulates-the-performance-

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen