EARLY LIFE STAGE BOTTLENECKS OF CARNIVOROUS MOLLUSKS UNDER CAPTIVITY: A CHALLENGE FOR THEIR FARMING AND CONTRIBUTION TO SEAFOOD PRODUCTION

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Abbreviations: fatty acids (FA); triacylglicerides (TG); octopine dehydrogenase (ODH); lactate dehydrogenase (LDH); citrate synthase (CS); glucose-6-phosphate dehydrogenase (G6PD); ß-hydroxyacyl CoA dehydrogenase (HOAD), Recognition Receptors (PRRs),

Pathogen-Associated Molecular Patterns (PAMP), Territorial use rights in fisheries (TURFs), Toll-like receptors (TLRs)

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ABSTRACT

This work brings together the view of different specialists in the areas of larviculture, physiology, ecology, nutrition and animal health, regarding how to deal with the aquaculture farming of species with complex life cycles in a multidisciplinary way, using as models the octopus and the muricid *C. concholepas*, with the aim of reducing the gap between the experimental and the industrial culture of species that are relevant for the diversification of aquaculture, particularly in Chile. Although these species are similar in their difficulty to reach the terminal planktonic phase prior to juvenile, they differ in the bottlenecks they have to overcome to reach it. Relevant aspects of study to achieve juvenile production from early life stages rearing, whether for repopulation or for ongrowing, are:

1) Replacement or supplementation of live diets with inert diets to achieve significant survival values over the first stages of life.

2) Physiological approaches to establish cultivation conditions evaluating the individual responses to several rearing conditions, specially the interaction between temperature, dissolved oxygen and acidity. Studies of urgent character due to the global warming scenario.

3) Genomic studies associated with the effect of ontogenetic development, environment, health and nutrition on gene expression to understand, in an integrated way, the key processes for the development and growth of immature stages.

4) Studies on the control of reproduction, the quality control of the ova, the genetic structure of reproductive populations, and the characterization of diseases are also necessary to achieve efficient hatchery technologies.

INTRODUCTION

The fishery of marine resources at global level are having serious impacts (FAO 2016), with potential negative effects in the medium and long terms, on the availability of these resources of high-quality protein for humans. During the last decades, a reduction in fisheries captures from some carnivorous mollusks with high market values have been reported (FAO 2016). In this regard, aquaculture can compensate and mitigate the fisheries effects. For example, by restocking natural banks with juveniles produced under controlled hatchery conditions to maintain or increase the production of endangered fishery species, supporting the development of coastal communities that have traditionally relied on these resources, as well as providing high quality food. This strategy requires an in-depth study of the management conditions of these juveniles in the wild to achieve a real restocking effect. Alternatively, the production of ongrowing up to commercial size of overexploited species, either on land or at sea, from juveniles produced in hatchery could partially or totally replace the fishery of that species. This challenge requires strategies to change artisanal fishermen, who are extractors, into small-scale growers.

In this regard, the Chilean and Peruvian predatory muricid gastropod *Concholepas concholepas*, locally known as “loco” (marketed abroad as “Chilean abalone” but it is a false abalone), shows an even more dramatic fishery pattern (Fig. 1) where the highest catches took place during the 80s followed by a collapse due to overfishing, and populations are still far from recovery in most places. Particularly, the Chilean government has emphasized the urgency of giving sustainability to artisanal fishery by producing an innovative strategy of restocking, based on hatchery production of juveniles to restock along Chilean coast.

Octopuses, like other benthic predatory mollusks, often have a complex life cycles that include indirect development through an immature planktonic stage prior to juveniles (merobenthic octopuses), which includes parental care of the eggs and, in some cases, very complicated feeding and territorial behaviors. Such characteristics make these species considerable vulnerable to overfishing. Therefore, in many populations around the world there is a risk of resource overexplotation if the fishing management practices do not improve. In this review, the two commercial species of octopus in Chile, *Octopus mimus* and *Enteroctopus megalocyathus*, are compared with another two species that have been thoroughly studied for their development in aquaculture at global level, *Octopus vulgaris* and *Octopus maya*, through the evaluation of their production of juveniles under controlled conditions.

Potentially, commercial production of these species under controlled conditions, or the re-stocking with aquaculture-produced juveniles, could contribute to satisfy the market demands, reducing the pressure on wild populations. Considering that these are carnivorous species, from an ecological and economic perspective it could be more appropriate to restock natural populations. This would allow both re-gaining a natural trophic food web balance, and providing more high quality seafood and livelihood opportunities for coastal small scale fisheries. Altough restocking presents important environmental challenges including the potential genetic disruption of natural populations, it may be one relevant solution to overexploited coastal fisheries (Bell et al 2008, De Silva, 2016).

Many research efforts have focused on the culture of different octopus species around the world, and the gasteropod *C. concholepas* in Chile. Despite the investment in infrastructure and research, the production of early life stages from these carnivorous mollusks is still the major bottleneck for their mass production (Villanueva et al. 2014; Manríquez & Castilla 2011).

The candidates for commercial aquaculture production proposed for protecting overexploited fishery worldwide include: *O. vulgaris*, *O. maya*, *O. mimus*, *E. megalocyathus*, and *C. concholepas.* The last three ones are found in Chile.

The common octopus*, O. vulgaris*, is a cosmopolitan merobenthic species that inhabits the tropical and subtropical coasts of the Atlantic, Indian, and Pacific Oceans and the Mediterranean (Sanchez et al. 2015). It inhabits the coastal waters with a vertical distribution from the surface and up to 200 meters from the coast (Sanchez et al. 2015). In nature, commercial size octopuses reach an average weight of 2 to 3 kg. *O. vulgaris* is the most demanded octopus species worldwide and the continuous reduction of wild captures makes imposible to sustain its fishery (FAO 2016, Fig.2). A wide range of artisanal gears for their catch can be found, including traps, pots and fyke nets in the Mediterranean Sea, that may constitute up to 30-45% of the captures in Spanish or Greek harbours (Tsangridis et al. 2002) and in Portugal (Pereira 1999). Thus, there is an increasing interest in the development and improvement of the culture techniques for *O. vulgaris* (Barnabé 1996; Iglesias et al. 2004, 2007; Socorro et al. 2005), but the high mortalities observed during the paralarvae stage strongly limited the development of commercial production of juveniles from this species.

The Mayan octopus (*Octopus maya*) is an endemic holobenthic species that develops in shallow waters during all stages of life, and with a restricted distribution into the continental shelf of Yucatán Peninsula located at the entrance of the Gulf of Mexico (Solís -Ramírez et al. 1997; Solis-Ramirez 1967; Voss & Solis-Ramirez 1966). This species inhabits the shallow waters from the Yucatan platform to a maximum depth of 60 m (Solís-Ramírez & Chávez 1986). They reach a maximum age of 12 to 18 months (Solís 1998), achieving up to 1 m total length (Rosas et al. 2013) and a maximum average weight of 3.5 Kg (Santos-Valencia & Re-regis 2000). With a production that exceeds the 20,000 ton/year (Fig.2), it is the most exploited *Octopus* species in Latin America (Rosas et al. 2014). Since this species have a direct development without the paralarvae stage, it is easier to start their nutrition research and feeding from the newly settled juveniles up to further stages.

Enteroctopus megalocyathus, the Patagonian red octopus, is a merobenthic species with planktonic paralarvae, inhabits the southern end of South America, in the shores of the Pacific and Atlantic oceans (Ibáñez & Chong 2008; Ibáñez et al. 2009). In Chile, it was caught under the name of "common octopus" along with the species *Octopus mimus*, resulting in an overfishing followed by a fishery closure between 2008 and 2011 (Fig.3). Currently, their fishery is affected by an annual reproductive fishing-ban between spring and summer. It is necessary to overcome the current bottleneck in the paralarvae stage to achieve the production of juveniles from paralarvae, either for ongrowing or restocking purposes (Uriarte & Farías, 2014).

Octopus mimus is also a merobenthic species distributed from the north of Peru (Tumbes: 3°34′S80°27′W) to the Central Region of Chile (Talcahuano: 36°43′S73°07′W), living in the rocky coastline and also at 30 m deep (Cortéz et al. 1995; Guerra et al. 1999; Olivares et al. 1996). This species provides the main octopus fisheries in Chile (70% of total landing with about 2200 ton. year^{-1}), and is vulnerable to overexploitation due to its high demand in international markets (Fig.3). The production of juveniles from paralarvae reared under controlled conditions has not been successful in this species (Zúñiga et al. 2014). A possibility to protect this species, considering its high fecundity, could be the permanent repopulation of planktonic paralarvae by means of a control and protection system of mature females in captivity, considering the vulnerability of the females either to predators or fishermen who extract them during the incubation period.

The muricid *C. concholepas* is an endemic species from the Southeast Pacific coast, distributed from tropical (Lobos Afuera Island, 6°S) to sub Antarctic habitats (Cape Horn 56°S), including Juan Fernández Island (Cárdenas et al. 2008). *C. concholepas* is a key-stone species component of rocky intertidal and subtidal ecosystems along the Chilean coast (Castilla, 1999) with high economic importance for the industry due to its high demand worldwide (Bustamante & Castilla 1987; Leiva & Castilla 2002). At present, the access to its fishery has been controlled and restricted, but in order to protect this and other species, and recover them for the future generations, territorial use rights in fisheries (TURFs) are granted to artisanal fishermen to enhance the sustainability of small-scale fisheries (González et al. 2006; Gelcich et al. 2017). However, these management plans for the resource do not seem to be enough to ensure its sustainability, thus encouraging the culture of this species. Although there has been significant progress on the understanding of various biology spects of *C. concholepas*, there are not standardized technologies yet that allow a full control over the life cycle in captivity (DiSalvo 1988; DiSalvo & Carriker 1994; Campos et al., 1994).

The production of early life stages of these five species has not been very successful due to high mortalities making difficult to achieve sufficient quantities of juveniles for commercial ongrowing or restoking (Table 1). Moreover, isolated efforts to develop the culture techniques required for each species have been insufficient and, with different degrees of success for each. Possibly, the exchange of information, coordinated research and joint actions could facilitate solving specific knowledge gaps to successfully develop and maintain early life stages.

Similarly, species with paralarvae/larvae stages show high mortalities that are associated to numerous factors related to the characteristics of the species in their natural ecosystems and that are very difficult to replicate under production conditions (Lee 2003; Villanueva & Norman 2008; Burnell & Allan 2009). A multidisciplinary approach is therefore required to solve these complex problems and obtain better early life stage performances. The homeostasis of these first stages of life is difficult to maintain under controlled conditions, especially during long incubation or rearing periods, to develop their tissues, organs and systems with the associated physiological changes to achieve a fully mature juvenile stage. Frequently, larval stages are the most vulnerable, showing high mortality rates (Pechenik et al. 1998; Cahu & Zambonino 2001; Lee 2003; Li & Leatherland 2012). Thus, a thoughtful knowledge of a species is required, from the early stages of life to the maturation process. This task involves the joint work of specialists on rearing technology, nutrition, physiology, molecular biology, health, among others, to standardize larvae culture to obtain juveniles for the on-growing up to commercial size organisms. For this reason, multidisciplinary knowledge has been the key for continuous innovation in aquaculture, where the synergy of different disciplines will enhance the production processes.

For all the reasons mentioned above, the culture of juveniles and even the culture of all the stages of the life cycle will be key for both productive sustainability and the adaptation to climate change of coastal communities that depend on complex benthic resources, such as octopus and *C. concholepas*. The aim of this work was to review the progress of different research lines addressing the rearing of the early and juvenile life stages of these species, as well as other similar species, to establish a prioritization on the research strategies to achieve as soon as possible the production of juveniles under controlled conditions of cultivation.

1. – Current knowledge on culturing early stages

1.1. The common octopus *Octopus vulgaris*

The common octopus is the most studied and standardized species under conditions of controlled reproduction and paralarval culture (Iglesias et al. 2014). The single spawning of this species may occur at different times of the year. Females spawn between 100,000 and 500,000 eggs measuring an average of 2.7mm (Mangold & Boletzky 1973; Villanueva &Norman 2008), forming several clusters of about 700 eggs each (Villanueva et al. 1994). During the spawning and egg care the female stops eating, usually dying from anorexia at the end of embryonic development, as in all octopus species (Mangold 1983). Upon completion of the embryonic development, the planktonic paralarvae hatch, and this stage lasts from nine to twelve weeks before adopting the benthic life (Villanueva et al., 2002; Villanueva & Norman 2008; Iglesias & Fuentes 2014).

The total length of paralarvae reaches between 2 to 3 mm, while the average dry weight of the newly hatched paralarvae is 0.36 mg (Moxica et al. 2002). They feed on the zooplankton found in the natural environment, mainly on different crustacean species (Roura et al. 2012; Lourenço et al. 2017). Under captivity, the massive paralarvae mortalities have hampered its commercial production (Iglesias et al. 2007, 2014) and thus, unappropriate feeding is a main cause that constrains standardization of paralarvae culture, although progress is being made with the use of new technologies (Garrido et al. 2016a). Nowadays, successful paralarval rearing is a bottleneck for the development of the culture of the species at industrial scale (Vidal et al. 2014; Villanueva et al. 2014; Garrido et al. 2016a). The brine shrimp *Artemia*, a typical live prey used in marine larval cultures, has incomplete nutritional quality for this species in terms of fatty acids and other micronutrients (Villanueva & Navarro 2003; Seixas et al. 2010; Estefanell et al. 2013; Navarro et al. 2014), thus producing massive mortalities in paralarval cultures. The most successful rearings and higher survival rates have been obtained using marine planktonic organisms like *Grapsus grapsus* zoeae, or in co-feeding schemes using *Artemia* and *Maja squinado* zoeae (Iglesias et al. 2004). Higher survival rates (31.5% at 40 dph) have been obtained using *M. squinado* zoeae (Iglesias et al. 2004, 2014; Garrido et al. 2016a). Villanueva et al. (1994) also obtained relevant results with *Liocarcinus* and *Pagurus* (73% at 25 dph). However, rearing paralarvae using crustacean zoeae is impractical at commercial scale (Villanueva 1994). Until today it is difficult to successfully trespass the barrier of 30 days of paralarval culture (Berger 2010; García-García et al. 2014; Iglesias et al. 2004; Iglesias & Fuentes 2014, Garrido et al. 2017). In fact, the effects of different live preys (*Artemia* and crustacean zoeae) and/or *Artemia* enrichment protocols in the paralarval growth have been studied by using a meta- analysis approach by Garrido et al. (2016a). The study found statistical confirmation of the better suitability of crustacean zoeae with respect to *Artemia*, highlighing a beneficial effect of marine phospholipids which is probably related to the content of DHA and polar lipids in the food, pointing at the essential role of these lipid components in octopus paralarval physiology.

Globally, it can be concluded that FA profiles change dramatically with age, feeding and husbandry conditions (Navarro et al. 2014; Garrido et al. 2016b). Under culture, paralarval development is associated to a decline in long-chain polyunsaturated FA paralleled by an increase in linolenic acid (18: 3n-3), which is very abundant in certain types of *Artemia*, and accumulates preferably in their neutral lipids (Viciano et al. 2011, Navarro et al. 2014). There is also an increase in total lipids during development, associated to an accretion of triglycerides (TG) from the lipid-rich *Artemia* (Navarro & Villanueva 2000, 2003; Navarro et al. 2014). Besides, FA profiles have been associated to variations in origin (wild stocks) or feed differences, and the study of biomarkers of stress and condition under different feeding scenarios reveal that initial size (dry weight) and metabolic status of paralarvae are probably related to the geographical origin of the broodstock (Garrido et al. 2017). Although an undeniable correlation between biomarkers of metabolism and stress, and the diet supplied to paralarvae has been found (Varó et al. 2013, Varó et al. 2017), potential overriding effects of unknown factors other than the quality and quantity of the lipid composition may play a role. To further deepen on the subject, the nutritional stress of paralarvae under culture conditions is being studied by means of proteomic approaches, with the aim of defining "proteomes" of the paralarvae associated to both the quantity and quality of their food (Garrido et al. 2017).

A substantial effort has been recently carried out to clarify the nutritional requirements of the *O. vulgaris* from the viewpoint of the essential lipids. The molecular and functional characterization of key enzymes in fatty acid (FA) metabolism has been undertaken (Monroig et al. 2012a, 2012b, 2017). A stearoyl-CoA desaturase (Scd) exhibiting Δ9 activity, and a fatty acyl desaturase (Fad) exhibiting Δ5-desaturase activity on both saturated and polyunsaturated fatty acyl substrates have been isolated (Monroig et al. 2012b; 2017). However, *O. vulgaris* seems to be unable to perform Δ6 or Δ8 desaturations, indicating that EPA and ARA must be indeed dietary essential FA for the species. Besides, the Scd is potentially implicated in the biosynthesis of NMI FA (Monroig et al 2017).

With regard to the elongases (Elovl), an Elovl with high homology to the vertebrate Elovl2 and Elovl5, consequently named “Elovl2/5” has been characterized (Monroig et al. 2012a). The octopus Elovl2/5 efficiently elongates C18 and C20 PUFA substrates, but has no activity towards C22 PUFA, being not itself capable of performing the elongation steps required for DHA (Sprecher, 2000). Finally, an Elovl4-like elongase involved in the biosynthesis of very long-chain (>C24) PUFA, has also been cloned and functionally characterized (Monroig et al. 2017).

The results outlined above, pointing at the essentiality of PUFA for the species, are consistent and complementary with those performed "*in vivo*" on hatchlings by Reis and co-workers (Reis et al. 2014, 2016). Using radio tracer studies, these authors have shown that the esterification pattern of radiolabeled FA substrates is highly specific, with DHA and C18FA being preferably esterified into phosphatidylcholine (PC), and ARA and EPA into phosphatidylethanolamine (PE). Besides, ARA seems to be a key FA for the species (Reis et al. 2015; Estefanell et al. 2017; Lourenço et al. 2017). Radio tracer studies show that ARA it is the most efficiently incorporated FA, as well as the least transformed, and its highest content is found in PE rather than PI (as is usual in other species), irrespective of the phospholipid in which it was initially supplied (either PC or PE) (Reis et al. 2016). Thus, the nutritional requirements of *O. vulgaris* hatchlings in terms of FA seem to be highly specific and so, taken as a whole, contradicting former results (Seixas et al. 2010), these results provide evidence confirming that certain PUFA (i.e. ARA, DHA) are essential nutrients that must be supplied in the diets of *O. vulgaris* paralarvae to guarantee survival and normal development.

1.2. The Mayan octopus, *Octopus maya*

The Mayan octopus spawns only once in its lifetime just like other octopuses, between February and March or July (Arreguín-Sánchez et al. 2000). Reproduction takes place near the coast where each female lays on hard and rocky bottoms between 1,500 and 2,000 large eggs of 17 mm long and 4.5 mm wide (Solís-Ramírez 1967; Solís-Ramírez & Chávez 1986). The embryonic development of this species varies from 50-65 days (depending on the water temperature) in the wild (Solís-Ramírez 1967). Hatched embryos seem to be similar to adults and become active predators almost immediately, and in turn, they occupy the same substrate as its conspecific (Arreguín-Sánchez et al. 2000). Development concludes during the post-embryonic stage of early juvenile (around 20d after hatch at 25°C), the digestive gland matures and the mechanisms of selective predation are activated (Martinez et al. 2011; Moguel et al. 2010; Portela et al. 2014).

The conditions for pre-ongrowing, ongrowing and reproduction in a controlled environment have already been established for *O. maya* (Rosas et al. 2014). In those studies a culture density of 25 hatchlings m^{-2} was recommended (Rosas et al., 2014) when animals are maintained in 22 m^2 external ponds (Domingues et al., 2012). In those ponds, hatchlings 1 d old are fed with amphipods that are offered as initial preys, along with an artificial diet designed for this species (Martínez et al., 2014). In a semi-pilot scale of production (ponds of 22 m^2), a 50% survival can be obtained when hatchlings of *O. maya* with 100 mg ww are cultivated until 250g ww. That crop weight can be reached in 4 -5 months at 26-28°C. A series of nutritional studies under controlled conditions have shown that the Mayan octopus is unable to digest alginate in formulated diets and they have a low protein digestibility of protein processed at high temperatures near to 100ºC (Domíngues et al. 2007; Rosas et al. 2007; Rosas et al., 2011; Rosas et al. 2008; Rosas et al. 2013). Currently, research on the digestive physiology of *O. maya*, *O. vulgaris*, *O. mimus*, and *E. megalocyathus* have shown that these species share similar limitations regarding the digestion of some ingredients (Farías et al. 2010; García-Garrido et al. 2010; Domíngues et al. 2010; García-Garrido et al. 2011a,b; Cerezo et al. 2012). In this sense, specific processed food has been successfully developed for the ongrowing of this species (Martínez et al. 2014).

1.3. The Patagonian red octopus, ***Enteroctopus megalocyathus*** (Gould, 1852)

The fecundity from this species under controlled conditions reaches up to 5,500 ± 948 eggs per female, these eggs measure between 7.0 to 11.5 mm and they spend 4 to 5 months in embryonic development until hatching (Uriarte et al. 2014), only 75% of the eggs are successfully fertilized (Uriarte & Farías 2014). The paralarval development to benthic settlement takes between 90 to 114 days (Uriarte & Farías 2014). The longer incubation and extended paralarval rearing are the most relevant differences between the Patagonian red octopus with the other octopus species of this review. However, during the ongrowing period this coldwater species showed similar growth rate to the tropical *O. maya* (Farías et al. 2009). Therefore, Patagonian red octopus takes two and a half years to complete the period from the reproductive phase of conditioning, through the stages of incubation of eggs, paralarval culture, settlement, and culture of juveniles until adult stages with an average weight of 2.5 kg (Uriarte & Farías 2014). It has been shown that during the egg incubation, the absence of the female does not affect the offspring, while the incubation period can decreased in 25 days by managing the temperature, improving their performance (Uriarte et al. 2016). Still, the main limiting factor is the survival of paralarvae, with maximum values of 0.46% of total incubated eggs. This period is critical and it has been the subject of numerous studies, describing the requirements for preferential and critical temperatures in early paralarvae (Uriarte et al. 2017), and feeding with live enriched *Artemia* (Farías et al. 2016). Recent nutritional studies have shown that vitamins like ascorbic acid and alpha tocopherol contained in the yolk are used during embryo development and their levels increase in paralarvae from hatching depending on the exogenous feed (Hernández et al. 2017). Nutritional knowledge on this species should be the starting point to formulate inert diets that could improve survival by either substituting or complementing the *Artemia* based diet.

Several studies have been conducted on ongrowing juveniles regarding the protein/energy ratio in diets (Gutiérrez et al. 2015), digestibility of different ingredients and different formulated diets (Farías et al. 2010; Martínez-Montaño et al. 2016). In adults the presence of Δ 5 and Δ 9 desaturase have been studied (Manríquez et al. 2013), indicating its importance in this coldwater species that are able to synthesize the long chain polyunsaturated fatty acids (LC-PUFAs) in the presence of its precursors in the diet.

1.4. The Eastern South Pacific octopus, *Octopus mimus*

The controlled conditions for broodstock in captivity, allows the females to spawn at any time of the year (Zúñiga et al. 2014). To date, all studies made on *O. mimus* used wild and inseminated females collected from a depth of 20 m off the coast of Antofagasta, Chile (23º lat.S.). Single spawn contains up to 400,000 eggs, measuring an average length of 2.14 mm. After 55 -60 d embryos hatch as planktonic paralarvae with a mantle length of 2.21 ±0.07 mm (Baltazar et al. 2000; Warnke 1999; Zuñiga et al., 2014). Even if the female starvation still promotes the gonadal development, the female fecundity may decrease, whereas the quality of progeny in terms of paralarvae survival is not affected in comparison with fed females (Olivares unpublished results). The nutritional requirements of broodstock females and its impact on paralarval quality is still unknown due to the limited period of paralarvae survival (7 days). At this age, the yolk reserves have been completely exhausted, because the internal yolk sac has been consumed between the fourth and fifth day after hatching (Zúñiga et al. 2014). Therefore, it would be necessary to study the reproductive success at different temperatures evaluating the efficient use of nutrients from the food reserve. Although this species is considered to have a good potential for aquaculture, there are still significant challenges ahead to understand its physiology and nutrition to complete the paralarval culture up to the juvenile stage (Linares et al. 2015; Olivares et al. 1996; Uriarte et al. 2012; Zúñiga et al. 1995; Zúñiga et al. 2013).

1.5 The muricid gastropod *Concholepas concholepas*, loco or false abalone

C. concholepas are dioecious organisms with internal fertilization, and they differentiate from octopuses by being able to reproduce many times in their lifetime. During breeding seasons, these whelks form large aggregations of several adults (Castilla & Cancino 1976; Manríquez & Castilla 2001). Fecunded females lay their capsules in the low rocky intertidal and shallow subtidal areas (Manríquez & Castilla 2001). A single female can deposit ovicapsules for several weeks, where each capsule may contains between 2,000 and 10,000 eggs (Ramorino 1975; Castilla & Cancino1976; Manríquez & Castilla 2001). Unlike other muricid gastropods, in this species the encapsulated embryos do not have ovophagia or adelphophagia (Gallardo 1973). Spawning times vary with latitude and temperature, being possible to find capsules at the bottom of the rocky intertidal and/or sub tidal from May to December (Fernández et al. 2007). In general, large females lay larger capsules containing a higher number of larvae (Manríquez & Castilla 2001), although significant differences in the average number of larvae per unit area of capsule (mm^2) have been found along the latitudinal gradient, being significantly lower in the northern range of distribution of the species (Fernández et al. 2007). At the end of the period of embryonic development, veliger larvae are released from the capsules and stay for several months in the water column before settling (Manríquez & Castilla 2001). In some cases, the larvae can be released up to 12 months as reported in southern Chile fjords and inland seas (Molinet et al. 2005).

The competent larvae, ready to complete metamorphosis, is observed between autumn and winter in southern Chile (Region XI, 45°lat. S; Molinet et al. 2005, 2008). The metamorphosis occurs when competent larvae reach a size of 1.4 to 1.7 mm, and occupies the surface layer of the water column or neustonic layer (Di Salvo 1998, Molinet et al. 2006). After metamorphosis, the settlement process occurs in the rocky intertidal zone, specifically in tide pools (Reyes & Moreno 1990), as well as in the sub tidal zone (Arias 1991; Stotz et al. 1991). The settlement in southern Chile occurs in winter (Moreno et al. 1993; Molinet et al. 2005, 2008) in the low tide area of the splash zone, associated with the presence of sea anemones. Individual marking has shown that at 8 mm in total length, the post-larval juveniles can move towards the mid and upper intertidal (Molinet et al. 2005, 2008). Juvenile growth from 1 to 6 months after metamorphosis, animal growth range from 0.022 mm day^{-1} up to 0.058 mm day^{-1} in the Region of Los Ríos (39° lat. S; Reyes & Moreno 1990), while in the Aysén Region (45° lat. S.) these values reach up to 3 mm month^{-1} (Molinet et al. 2006). In experimental cage systems in the Aysén Region, with densities of 9 newly settled juveniles m^{-2}, juveniles reach 3.5 cm in total length during the first year (Molinet et al. 2005), while at the end of the second year it is possible to obtain reproductive adults sizes from 7 to 9 cm. On the other hand, in Valparaíso Region (33° lat. S.) there are cage systems that have reported growths that would allow reaching the commercial size of 10 cm in 1.65 years (Manríquez et al., 2008). The obtention of harvest-sized adults within two years shows that it is a resource growing more than true abalones (Pérez, 2010), therefore it has a potential for aquaculture. Under controlled laboratory conditions it is possible to achieve reproductive conditioning for oviposition, hatching of veliger larvae, and even larviculture to produce competent larvae to the further production of juveniles for ongrowing (DiSalvo, 1988; DiSalvo & Carriker 1994; Campos et al. 1994, Manríquez & Castilla 2011). According to the laboratory experiences with this species two critical problems need to be solved to upscale to commercial production of juveniles: a) Low larval survival rates until their settlement and metamorphosis; b)

Insufficient quality and quantity of live prey to feed the post-metamorphic juveniles from settlement to juvenile size of 2 cm.

2.- Larviculture challenges

2.1. Optimal nutrition for broodstock and early life stages

Given the different nutritional requirements of early life stages, pre-ongrowing and breeding stages, it is necessary to carry out experimentation based on different types of food in interaction with various environmental, demographic and developmental variables. Broodstock nutrition in octopuses has been tested by the chemometric analysis of eggs, embryos and larvae to generally define the characteristics that broodstock food should have (Navarro & Villanueva 2003, Hormiga et al. 2010, Farías et al. 2011, Navarro et al. 2014; Caamal-Monsreal et al., 2015; Quintana et al. 2015). However, fresh diets, mainly crabs, are still used as the best choice during the reproductive conditioning, a fact that needs to be changed to sustain its culture. The spawn quality was generally correlated to parental diets containing high quality protein, lipid clases like EPA, DHA, phosphatidylethanolamine, phosphatidylcholine, cholesterol and triacylglycerol. In this sense, Tercero et al., (2015) demonstrated that high quality fatty acids included in an elaborated diet improve the spawns and embryo growth and survival of *O. maya* at hatching.

There are no reports of nutrition studies on the reproductive conditioning and larval culture of *C. concholepas*. However, Vargas et al. (2006) examine the spectrum of food sizes and food types consumed by the larvae of this species, showing that veligers are omnivorous grazers, incorporating significant fractions of heterotrophs in their diets. Newly hatched *C.*

concholepas larvae also consumed picoplankton cells, while competent larvae of this species ingested mostly the largest phytoplankton cells and heterotrophic protozoans.

The nutrition and feeding of paralarvae are similar to that of marine fish larvae, and advances have been made in the development of larval food for the latter (Kolkovsky 2013; Takeuchi & Haga 2015, Olmos et al. 2017). A recent progress on this field has been the replacement of *Artemia*, the most commonly used live food in marine larviculture, by copepods. Even if copepods are somehow superior than *Artemia* in their essential nutrient content, at present their culture has not been adequately controlled, so far without achieving success in octopus paralarvae (Roura et al. 2012). Nevertheless, in *E. megalocyathus*, the paralarval development using *Artemia* has been successfully performed (Uriarte & Farías 2014), although a co-feeding with formulated diets or other live preys is required to improve survival until settlement and metamorphosis stages in different cephalopod species (Olmos et al. 2017).

In a recent study using molecular tools, Olmos et al. (2017) describe the identity of octopus and squid paralarvae prey in their natural environment. The authors identified that the diet of wild *O. vulgaris* paralarvae is composed by decapods, copepods, euphausiids, amphipods, achinoderms, mollusks and hydroids, with seasonal and space variability. However, under control conditions, despite all the current efforts for the development of marine larvae feed, live food has not been entirely replaced by formulated feeds for marine species (Dhont & Dierckens 2013). The main difficulty for *Artemia* replacement, as well as any other live food, is their high content of free amino acids, peptides and phospholipids, all easily leachable compounds. Thus, in order to be replaced and delivered correctly they have to be encapsulated (Kolkovski et al. 2009). However, the microcapsules are generally made of complex carbohydrates or lipids with a low digestibility that reduces their use in marine larvae with an immature digestive tract (Cahu & Zambonino 2001). Nontheless, the pre-digestion process described in octopus paralarvae (Nande et al. 2017) and early juveniles of *C. concholepas* before food intake in comparison to fish larvae, can be seen as an advantage for the use of microcapsules (Ronnestad & Morais 2008). In this way, the generation of formulated food for octopus paralarvae may apply with innovative strategies of molecular assembly to produce technologically advanced feeds. These feeds must combine thermodynamic aspects of the molecules to be assembled (thermal stability, photosensitivity, water affinity, stability and dynamics of molecular aggregates with different physicochemical characteristics, etc.), with kinetic aspects (hydration status, hydration rate, leaching out of the formulations, controlled or sustained release of nutrients over time, etc.). In addition, they should consider physiological aspects of the early larval stages including an inmature digestive system that hinder the absorption and bioavailability of nutrients with high molecular weight, such as proteins, or hydrophobic compounds, such as triglycerides and cholesterol (Ronnestad & Marais 2008; Tocher et al. 2008). Therefore, the most technologically advanced formulations will consider physicochemical activity with enzymatic activity, biocompatibility and non-toxicity ingredients, which can also regulate the color, shape and size to resemble the prey preferred by larvae. The particle kinetic, dynamic behavior (buoyancy and trajectory) and palatability of the feed are decisive to promote larval intake and requires a deep knowledge of the physiology of the species at different developmental stages. The challenges that these scientific and technological developments represent should be addressed with the aid of new analytical technologies, and knowledge in the management of the molecular interactions to enable the molecule assemblies to meet various and simultaneous requirements (Binsi et al. 2017). Undoubtedly, the achievement of objectives associated with the generation of formulated diets for marine larviculture requires a multidisciplinary approach to generate significant advances in larval/paralarval feeding technology. Besides, there are behavioral factors linked to nutrition which also affect the survival and growth of these immature stages of development, highlighting cannibalism mainly when the feed/prey does not fulfill the requirements, and during the first days of juvenile life in both octopuses (Moguel et al. 2010, Martínez et al. 2011, Miranda et al. 2011) and *C. concholepas* (Manríquez et al 2008). However, cannibalism could be triggered by stress and not necessarily by nutrition problems (Espinoza et al. 2017a). Starvation studies in paralarvae have highlighted the importance of catabolism of fatty acids and amino acids for energetic purposes (Speers-Roesch et al. 2016; Espinoza et al. 2017b).

Studies carried out on nutrition of octopus juveniles to establish the digestive dynamics and the effects of environmental variables, primarily temperature and dissolved oxygen, have shown which culture conditions and types of food are more suitable for the different species. Energy balance studies conducted in *E. megalocyathus* and *O. maya* suggest that cold water species could be more efficient in transforming the energy intake than the tropical species, partly explaining why *E. megalocyathus* reaches larger sizes than *O. maya* (Farías et al. 2009).

Digestive physiology studies have shown how octopuses digest, absorb and assimilate nutrients from food. For example in *O. mimus* and *O. maya* the digestion is carried out in two stages: the first one is characterized by the transit of soluble nutrients to the hepatopancreas that are immediately transported to muscle to be assimilated. The second stage include the digestive process of more complex nutrients, like those derived from myofibrillar protein, that require being broken into the stomach with a high enzyme activity. When *O. mimus* (temperate habitat) and *O. maya* (tropical habitat) were compared, it was evident that the timing of the digestive process and the type of digestion changed with the species, being more important the digestion of lipids in the case of temperate species and of protein in tropical ones (Gallardo et al. 2017). This process affects the content of AA in blood and the synthesis of complex molecules in the muscle (glycogen and protein), indicating the importance of soluble nutrients and complex nutrients in the design of complete feeds for these species (Martínez et al. 2012, 2014).

Until now, the number of nutritional studies on octopus paralarvae and *C. concholepas* larvae are limited compared to nutrition studies on juvenile octopus, probably due to the lack of success due to the rejection of inert feeds by the paralarvae. However, as stated before, the bioavailability of nutrients in paralarvae are mandatory to estimate the absorption capability of nutrients and its retention (protein accretion). Reis et al. (2014, 2015) studied the composition and metabolism of fatty acids using radioactively labeled molecules in *Octopus vulgaris* and *Sepia officinalis*. In particular, they concluded that LC-PUFAs and phospholipid must be considered essential dietary nutrients in *O. vulgaris* hatchlings. Later on, Reis et al. (2016) found that cuttlefish hatchlings may have the capability for the first steps in the biosynthesis of ARA and EPA from the 18:2n-6 and 18:2n-3 as precursors; also suggesting the presence of desaturases. The use of radioactive ^{14}C molecules requires particular protocols, being difficult sometimes to practice in aquatic environment laboratories. However, the use of stable isotopes in bulk makes it possible to trace the food or feed from the origin to its destination using the isotopic value of the diet. This can be done by using in bulk (with the whole sample) or by Compound Specific Stable Isotopes by searching per AA or fatty acids (FA). Instead, from bulk samples and using mix models, it will be possible to comparatively study the assimilation per AA (Martínez del Río et al. 2009). In larvae nutrition, it is possible to track the contribution of nutrients from live and inert feeds regarding its retention as growth through the use of stable isotopes (δ13C and δ15N), according to Peters et al. (2012). Furthermore, when various nutrient sources or processes are being employed in the diet, the stable isotopes can be efficiently used to directly track its differential retention in larvae food (Le Vay & Gamboa-Delgado 2011). By using the stable isotopes of δ15N and δ13C enrichment, it is possible to establish the nutrient requirements compared to those that are being used as energy instead of growth (Martínez del Río & Wolf 2005), even for larval diets (Gamboa-Delgado et al. 2008). Continuous sampling of larvae for bulk analysis will allow the estimation of the protein turnover rate caused by different food or feeds, besides the environmental controlled conditions such as temperatures. This kind of studies will be usefull for tracking the nutrients in a more precise way (Martínez del Río et al. 2009). Using different nutrient sources or inert microencapsulates composition will allow the measurement of digestibility success from microcapsules or different inert food processes. The retention efficiency from the different sources will be established to provide information and design the food needed per species throughout the entire stages of culture.

2.2. Environmental effects: Climate Change

Climate change, in addition to distribution and reproductive behavior, may reduce juvenile recruitment. The early stages of theses species have been characterized by the narrowest ranges of thermal tolerance (Pöertner & Farrell 2008). Besides, as marine carnivorous species, their natural preys are being also affected by the climate change making them locally or seasonally not available (Pöertner & Farrell 2008, Noyola et al. 2015), a risk that could be minimized by producing juveniles for restocking under culture conditions.

Some authors have shown that climate change could produce a mismatch between the time of larval release and food availability in the plankton with consequences for reproductive phenologies in the north Atlantic and North Sea, affecting the recruitment processes in fish and invertebrates (Richardson 2008, Genner et al. 2010, Poloczanska et al. 2016), a comparable situation than can be expected for other latitudes and regions. Therefore, the study of the climate change effect is needed to understand the biology of theses species and its interaction with environmental factors, at least until the species are totally domesticated. Further interdisciplinary and synergistic approaches are needed to enhance the knowledge. As mentioned earlier, species with complex life cycles undergo critical processes when going from one ontogenetic stage to another, representing bottlenecks that are especially sensitive to environmental factors. Remarkable critical processes are fertilization, spawning, hatching and metamorphosis. In aquaculture, these critical processes take place in the Hatchery where environmental conditions are controlled. However, attempting to establish a rearing or cultivation protocol setting values for environmental parameters should consider adaptation from natural environmental levels. When this information from nature is scarce, physiological approaches to establish cultivation conditions are particularly helpful evaluating the individual responses to several rearing conditions. Special attention should be paid to the interaction between different environmental factors since many of them show synergistic effects, such as the combination of temperature, salinity and dissolved oxygen. Therefore, the studies performed on cephalopods' rearing represent a valuable tool to anticipate the effects of climate change on both wild populations and potential cephalopod farming (Repolho et a. 2014).

Studies conducted throughout the life cycle of *O. maya* have reported it is high temperature sensitive, observing that embryos do not develop when temperature is higher than 27°C (Caamal-Monsreal et al. 2016). That result was later explained as an inability of embryos to neutralize the radical oxygen species (ROS) produced under high temperatures (Sánchez-

García et al., 2017). Also, it was observed that *O. maya* juveniles present a maximum growth rate at temperatures of up to 26°C, suggesting that a thermal limit could exist around that temperature in this octopus species (Noyola et al., 2013). In females of *O. maya* it was also noted that animals do not spawn when the temperature is higher than 27°C (Juárez et al. 2015). In that study was also observed that embryos and offspring from temperature stressed females are smaller than those from non-stressed females, denoting the epigenetic effect of temperature on offspring characteristics (Juárez et al. 2016). This could also be due to the higher energy expenditure from females exposed to higher temperatures being unable to store sufficient reserves. In sub-polar species such as *E. megalocyathus* it has been observed that it is possible to increase embryo growth up to 15°C (Uriarte et al. 2016), and the thermal tolerance of paralarvae can be modulated by the acclimation temperature between 6 and 18°C, with paralarvae showing little tolerance at these extreme temperatures and a high mortality at 18 º C (Uriarte et al. 2017). In *C. concholepas* it is highly probable that temperature might be playing a preponderant role controlling the biochemical reactions to determine the performance of these organisms during embryonic development (Fernández et al. 2007).

The increasing content of CO_2 in the athmosphere and in the marine environment causing acidification of the oceans will have negative effects on the overall physiology of the species (Lardies et al. 2014), especially mollusks and other invertebrates with exoskeleton. A fact that can also be detrimental for eggs and early developmental stages, and the control of pH in laboratory conditions could be most relevant.

2.3. Reproductive physiology and gamete quality

The reproductive physiology of these carnivorous mollusks is not well understood. During gonadal maturation, oocytes accumulate proteins and lipids, determining the quality of the egg and, in turn, a better survival (Ibáñez et al. 2008; Caamal-Monsreal, et al., 2013, Tercero et al., 2015). In fish, vitellogenesis is the most studied process. During this phase the lipoprotein vitellogenin is incorporated into the oocyte, being stored as proteolytic products (lipoviteline and phosphovitine) providing the AA and phospholipids for embryonic development. The expression of different types of vitellogenins during growth and maturation of the oocyte is considered a determinant of the final egg quality (Williams et al. 2014), whereas the amount of reserves is affected by the broodstock diet (Tocher et al. 2008, Tocher 2010). When *E. megalocyathus* and *O. maya* females received a restricted diet the fecundity was reduced without decreasing the oocyte quality (Farías et al. 2011, Caamal-Monsreal et al. 2015). In the *O. maya* study, it was also observed that females fed mixed diets apparently used energetic amino acids (AAs) and saturated and polyunsaturated fatty acids (FAs) in the diet to provide yolk with a combination of FAs and AAs that allowed hatchlings a better performance during the first days of culture. For that reason, mixed diets for *O. maya* females was recommended as a form to give to the animals the nutritional components to satisfy nutritional requirements for reproduction (Caamal-Monsreal et al., 2015).

An importance aspect in oocyte quality is the maternal transfer of innate immunity system, as in the case of vertebrates (Zhang et al. 2013; Li & Leatherland 2012). Even if this had not been tested on the species here presented, studying this aspect will be relevant.

According to Zhang et al. (2013) the vitellogenin, lipoviteline and phosvitine also have antimicrobial activity, and besides their nutritional role these lipoproteins may contribute to defend the organism, a subject that needs further research for the development of larviculture. On the other hand, in fish the maturation of the oocyte is synchronized by sex steroids, which include maturation inducers. These hormones provide information on the maturity status of females, allowing to program diets with special nutrients to avoid damages in the process of vitellogenesis or poor quality oocytes (Hachero et al. 2007). Variations in progesterone (P4) and testosterone (T) levels in the gonad of *Octopus maya* were investigated by radioimmunoassays and regarding four gonad maturation stages (GMS) and the reproductive cycle (Avila-Poveda et al., 2015). According to the GMS and the maturity indices, the reproductive season of *O. maya* from Yucatan occurred from February to June. In females, P4 and T displayed the same pattern; in contrast, P4 and T in male gonads displayed a different pattern, where T concentrations were relatively stable throughout the study. In that study was showed that gonadal P4 levels were elevated during gonadal maturation stage III (GMS-III) and GMS-IV (i.e. periods of vitellogenic oocytes), where the characteristic aspect is an ovary with very high oocyte diameters, with the primary follicle cells deeply infolded in the ooplasm for yolk synthesis. These results suggested a synchrony between P4 and the process of folliculogenesis, and in turn, vitellogenesis. In other study, Avila-Poveda et al (2016) found that synchrony is regulated seasonally suggesting that, as expected, natural thermal fluctuations are the key environmental factor modulating the reproduction of this octopus species (Angeles-González et al., 2017).

Since octopus species are semelparous, reproducing only once in their lives (Rocha et al. 2001), it would be critical to differentiate on time the moment when the reproductive period will take place, to adopt adequate decisions depending on the aim of the production.

Spermatogenesis is a cyclical process strongly affected by temperature, with seasonal variations linked to environmental signals in species of high latitudes (Schultz et al. 2010). Therefore, the spermatogenic factors that can alter the fecundity and survival of the embryos of these species must be studied. It is necessary to establish whether spermatogenesis has a complete development just prior to the females spawning, as is the case of fish (Campbell et al. 2003). Results obtained in *O. maya* females exposed at high temperatures indicated that animals maintained above 27°C had less fecunded eggs, suggesting that temperature could affect the viavility of the sperms stored in oviducal gland of this octopus species (Juárez et al., 2015).

On the other hand, knowing the plasma level of steroid hormones during male gonadal maturation would provide information on the increase peaks associated with the spawning season (Schultz et al. 2010), which is especially relevant in semelparous species such as octopus, in order to handle the organisms according to ongrowing or breeding purposes. Blood samples could be obtained from the cephalic aorta (following Castellanos- Martinez et al., 2014) but it will be necessary to develop molecular tools for detecting male and female hormones from available samples without sacrificing octopuses (Larson & Anderson 2010).

2.4. Genetic characteristics

Knowledge on the population genetical structure is essential to develop zootecnology practices to maintain the genetic variability and the biological adequacy of organisms (Toro et al. 2004a; Toro & González 2009; Astorga 2012; Sykes et al. 2017) for broodstock selection. This information will be helpful for the selection of stocks, for mass production in the laboratory, and to follow programs for genetical improvement (Toro et al. 2004b). The mass production of juveniles requires control over the complete life cycle of any organisms to allow the application of conventional breeding programs using genes that influence the the desired traits (body weight, genetic resistance to diseases, etc) (Martínez 2012; Toro & Oyarzún 2012). However, at present, it is not possible to perform in-breeding programs on octopus species with paralarval stage or *C. concholepas* because F1 has not been achieved. As a starting point, it will be important to characterize genetically the different wild groups or populations of these species, providing the basis for breeding at the beginning of the productive cycle in a controlled environment. Further control of adequate levels of genetic variability would allow a high response capacity of the offspring produced in the hatchery. On the contrary, the inbreeding will decrease the genetical variability affecting the overall performance of both species, octopuses and gastropods (Cianelli et al. 2013). Also, the genetic knowledge of the main mollusks produced by aquaculture is still insufficient (Astorga 2014). For this purpose, it is relevant to know the genetic diversity levels and to estimate the population genetic structure of these carnivorous mollusks, through the use of molecular markers.

DNA sequencing studies can also facilitate the development of molecular markers, such as single nucleotide polymorphic or single nucleotide polymorphisms (SNP) markers. These markers will then be available to achieve massive production of juveniles under controlled conditions, maintaining genetic variability while implementing breeding programs.

In *C. concholepas*, efforts have been made to improve the genetic knowledge at various levels. Firstly, there are research initiatives aiming to increase genetic tools for the species, including molecular markers such as microsatellites, transcriptome databases and the development of the complete mitochondrial genome (Cárdenas et al. 2007; Cárdenas et al. 2011; Núñez-Acuña et al. 2013). These types of studies have been useful in different ways, such as species distribution in relation to the current climatic and environmental changes (Hajibabaei et al. 2011) and distribution according to environmental changes (temperature, salinity, upwelling, community structure, among others). At present, there is evidence of isolation by distance between populations of *C. concholepas* (Garavelli et al. 2014, Cárdenas et al. 2016). The study of reproductive strategies of a commercially important species such as *C. concholepas* is key for the development of adequate conservation strategies for natural populations and is a milestone for aquaculture management. One of the main challenges is to determine a precise method to quantify reproductive success. A recent and simple method of paternity based on microsatellites for *C. concholepas* demonstrate the occurrence of multiple paternities in this species, showing that an ovigerous female can be fertilized by more than one male (Morales et al. 2016). This would imply that within a capsule mostly half brothers would be found, a mechanism of post-mating sexual selection that could allow the female to select only some sperm for fertilization (Morales et al. 2016). Ten polymorphic microsatellite DNA loci have been isolated from *O. maya* in an attempt to characterize the wild population that is used as a source of wild broodstock (Juárez et al., 2013). These loci represent the first microsatellites isolated from *O. maya* and are currently being employed in a number studies, that will allow the testing of hypotheses relating to phylogeography, population genetics and paternity in this species.

Multiple paternities in *O. vulgaris* is an issue that needs to be taken into account for population and conservation genetics, since it affects the effective population size (Sugg & Chesser 1994; Karl 2008). In addition, multiple paternity is relevant in the design of culture conditions and management to get an adequate male:female ratio for broodstock selection. Restocking strategies for the depleted octopus fisheries, considering a male-biased contribution will increase the effective size (Sugg & Chesser 1994). Multiple paternities can therefore maximise the genetic recombination with multiple males in a single reproductive event. The exhaustive egg care during reproduction will warrant a successful survival rate of genetically diverse offspring (Quinteiro et al. 2011). On the other hand, some studies on genetic diversity and population genetic structure in *O. vulgaris* from the Mediterranean Sea (De Luca 2016) have reported on differentiation and isolation between populations. Therefore, it is important to know what happens to these species in their distribution ranges, ensuring the adequate maintenance of variability levels in the natural populations or broodstock for aquaculture.

2.5. Pathology and diseases

Reports on histology and histopathology of cephalopods are scarce. The few available studies are mainly on the reproductive (López-Peraza et al. 2013; Avila-Poveda et al. 2016), digestive (Fernández-Gago et al. 2017; Roo et al. 2017), and central nervous (Wild et al. 2015) systems. A study of the ontogenetic development of circulatory, respiratory, nervous, and digestive systems in *O. vulgaris* paralarvae up to 75 dph has been recently conducted (Fernández-Ardiles et al. 2016). Similarly, there are publications on certain parasitic diseases of importance in cephalopods and their histopathology (Gestal et al. 1998, 2002a, 2002b, 2007, 2008, 2015; Pascual et al. 2007; Castellanos-Martínez et al. 2013a, 2013b, 2014; Castellanos-Martínez & Gestal 2013a, 2013b; García-Fernández 2016, 2017). Most emerging disease problems in mollusk are associated with species for which there is negligible reference material of normal and abnormal conditions. The farming of species with complex life cycle need to have health profiles established, as well as the environmental parameters associated with pathology at the tissue level (McGladdery et al. 2006; Aranguren & Figueras 2016). Few studies are available worldwide in this subject, and local information needs to be gathered to create a baseline from which the parameters of normality can be known in order to recognize the alterations that might be associated with disease and/or mortality events. In this way, it will be possible to establish cause/effect associations and take preventive or corrective measures to minimize losses, thus achieving a sustainable culture of species with complex life cycle.

3.- New metabolic and molecular research tools

Defining the associated genes expression with the different stages of the life cycle, and those associated with the ability to survive, develop and grow is a promising scenario to detect the metabolic pathways connected to survival success and the metamorphosis of larvae, among other relevant aspects (García-Cañas et al. 2010; Huan et al. 2012; Niu et al. 2016; García-Fernández et al. 2017; Varó et al. 2017). The study of these complexes interactions requires the development of advanced methods of analysis together with bioinformatics, proteomics and metabolomics to elaborate an adequate integration of the information provided by these disciplines (García-Cañas et al. 2010; Huan et al. 2012).

The overall study of the innate system by molecular tools will be important to identify possible recognition receptors for pathogens, such as the Toll-like receptors (TLRs) molecules that are responsible for this recognition and are located on the outer membrane and endosomes (Akira et al. 2006), or the Pattern Recognition Receptors (PRRs) that recognize Pathogen-Associated Molecular Patterns (PAMP). Functional analysis of new TLRs in aquatic animals is essential for the understanding of comparative immunology since recent reports have highlighted the additional functions of TLRs in adaptive immunity (Rauta et al. 2014). A large number of TLRs have been identified in various aquatic animals including fish, annelids, mollusks, arthropods, echinoderms and chordates (Kawai & Akira 2010). An atypical Toll-like receptor (HcToll-2) has been identified in the pearl mussel *Hyriopsis cumingii* (Ren et al. 2014). The HcToll-2 messenger (mRNA) was detected in the hepatopancreas and is overexpressed when challenged by *Escherichia coli* and *Staphylococcus aureus*. This expression is related to the expression of theromacin, which is an antimicrobial peptide (AMP) rich in cysteine. This research is the first report of a TLR that is both atypical and involved in antibacterial immunity through the induction of AMP expression (Ren et al. 2014), and further investigations are needed on the presence and activity of TLRs in mollusks at different stages of their life cycle, and also their relationship to the expression of antimicrobial peptides.

A recent study on *C. concholepas* described immune related genes based on a transcriptome study by exposing adult individuals to the pathogenic bacterium *Vibrio anguillarum* (Detree et al. 2017). The mapping of *C. concholepas* transcriptome revealed 61 immune-related transcripts related to immune function but also involved in numerous other biological processes such as oxidative stress, signal transduction, recognition of non-self, cell cycle and apoptosis. More studies like these are urgent to increase our understanding of the molecular basis immunity in these gastropods.

Besides, lysozyme and reactive oxygen species (ROS) are essential components of innate immunity of mollusks and part of their main antibacterial defense mechanisms. For example, lysozyme activity has been described in some species of cephalopods, mainly in *Sepia officinalis*, and it is associated with the integumentary, circulatory and epithelial systems, where its high levels of activity are probably related to the constant exposure to a large number of opportunistic water pathogens, as described for the skin of teleosts (Vidal et al. 2014; Ghafoori et al. 2014). Integumentary location of lysozyme is consistent with descriptions of bivalve mollusks, indicating the lysozyme activity role in the neutralisation of pathogenic agents after injuries or bacterial infections. While viruses, fungi, and bacteria can infect cephalopods, the latter cause the biggest problems in octopuses (Uriarte et al.

2011). In fact, cephalopods are susceptible to bacterial skin infections, as a result of secondary infection of wounds by opportunist pathogens (Castellanos-Martínez & Gestalt 2013), especially *Vibrio* spp., which is mainly found in epidermis and ulcerations. Haemocytes are part of the first defense barriers in these mollusk species due to phagocytosis of foreign pathogens and particles, which are then destroyed through production of enzymes such as lysozyme, as well as the presence of ROS and nitric oxide (NO) radicals (Gestal & Castellanos-Martínez 2015). All these defence strategies have recently been studied in *S. officinalis* and *O. vulgaris* (Gestal & Castellanos-Martínez 2015, Castellanos-Martínez et al. 2014a, 2014b), which opens other study strategy to determine the effect of diets, productive management, and stress, on the innate immunity of cephalopods.

Defining the baseline of health markers for this species at an early life stage should consider multiple indicators of the immune system, stress or infection. Attempts have being made to evaluate the potential markers for the oxidative stress as bio-indicators of the metabolic capabilities of *O. vulgaris* paralarvae, as well as route alterations that might have been produced due to the different nutritional treatments (Garrido et al. 2017). The methodologies exist, as reported by Speers-Roesch et al. (2016) and Capaz et al. (2017) in cuttlefish and Morales et al. (2017) in octopus, to assess the enzyme activities of intermediary metabolism such as: octopine dehydrogenase (ODH, exclusive of cephalopods) and lactate dehydrogenase (LDH), both indicative of anaerobic metabolism; citrate synthase (CS), indicative of aerobic metabolism; glucose-6-phosphate dehydrogenase (G6PD) as an indirect indicator of lipid synthesis; and ß-hydroxyacyl CoA dehydrogenase (HOAD), indicative of fatty acid oxidation. In a recent study was observed that antioxidant defense mechanisms of *O. maya* embryos were evident once the branchial hearts start their activity, reaching their maximum activity in stages XVIII, when the last turn of the embryo occurs (Sanchez-García, et al., 2017). The authors demonstrated that in embryos and early juveniles glutathione-s-transferase (GST), total glutathione (GSH) and catalase (CAT) can be used as indicators of the antioxidant condition of octopus embryos when they are exposed to temperatures higher than their thermal range.

The survival of marine invertebrates that are constantly exposed to pathogens rely exclusively on their robust innate immune system, which is mainly composed of hemocytes and antimicrobial peptides. In octopus, the haemocyanin has a clear antimicrobial activity, in addition to the primary function of oxygen transport (Martínez-Montaño et al. 2016). Thus, hemocyanin from *E. megalocyathus* inhibits growth of gram-negative bacteria containing profeniloxidase activity, and is responsible for the synthesis of melanin that has bacteriostatic, antiviral and wound repairing activities (Hernández et al. 2014a). Using a mussel *Choromytilus chorus* as a comparative model, it has also been reported a protein in the hemolymph with antimicrobial activity against both gram negative and positive bacteria. This antimicrobial activity is complementary to that of antimicrobial peptides present in the hemolymph of the Chilean mussel (Hernández et al. 2014b). The aforementioned examples allow to delineate the components of the innate immune system in marine invertebrates to contribute with the objectives of generating relevant knowledge on the innate immunity system along the whole life cycle of the species here presented.

Epigenetics and transcriptomics can determine the nutritional conditions that allow the best development and survival of the species through the nutrient/gene relationship, as well as the moment within the ontogenic development when diet can make important contributions to ensure the optimum use of the nutrients (Tammen et al. 2013, Zhang 2015). In species as complex as *C. concholepas*, involving a filtration/herbivory behavior prior to metamorphosis which is then replaced by a post-metamorphosis carnivorous habit, it is key to elucidate the timely sequence of nutrients that trigger the ontogenetic process. Epigenetic modifications encompass changes in gene expression profiles that occur without alterations in the genomic DNA sequence (Jaenisch & Bird 2003). Changes of the cellular metabolism affecting the chromatin modifying enzymes will make interesting subjects of study. The metabolites expressed will be the epigenetic marks for several physiological processes (Katada et al. 2012). In egg-laying organisms, for example fish, the gene coding for vitellogenin (vg) is transcribed in the female, but epigenetically it is quenched by hypermethylation of the vg promoter in males (Stolzenbach et al. 2014).

After the long-term effect on molecular markers of growth due to exposure to an acute nutritional stimulus in early stages of life was demonstrated, it is critical to determine the essential nutritional window during development, because it could have an impact on the permanent change of growth and post-hatching health. Currently, the patterns and function of methylation in invertebrates are unclear. In organisms with complex life cycles there are stages of high nutritional plasticity during embryogenesis and early larval development. Recently, it has been reported that in *O. vulgaris* the genome appears to be widely methylated and that the overall methylation pattern changes with development (Díaz-Freije et al. 2014). The strong morphological changes after hatching involve silencing and activation of genes where DNA methylation probably plays an important role.

4.- Sustainable management of mollusk resources

The sustainable development of aquaculture requires that this productive activity turn into an efficient, cost effective process carried out without affecting the environment. The culture of native species represents a challenge to produce commercial size organisms under sustainable basis to release the pressure of fishery.

The farming of a species with complex life cycle requires high efficiency at all production stages to reduce technical and economic vulnerability and to increase its resilience to climate change. This requires a multidisciplinary approach where biological, sociological and economical sciences collaborate to make this production possible.

The production of juveniles for restocking must ensure that the genetic diversity of the species is maintained. The knowledge of possible epigenetic changes generated by captivity and controlled production exists, and therefore measures should be taken if restocking programs are done with captive species in order to avoid a genetic alteration of species in their natural environment. For example, in the case of *C. concholepas*, it is considered the capture of wild competent larvae for rearing under controlled conditions and obtaining optimal juveniles to restocking.

5.- Link the juveniles production with ongrowing culture of marine carnivorous mollusks

The commercial ongrowing of the species discussed in this review can start with wild juveniles or with juveniles produced from hatchery, whether in ponds on land or in cages suspended on the sea. It is proposed that ongrowing technology of *C. concholepas* could be based on obtaining wild juveniles to restock TURFs areas by ranching. In the case of the common octopus *O. vulgaris* there is a significant advance in the development of cages, feeding strategies and nutritionally tested diets only for wild juveniles (Rey Méndez et al. 2003, García–García et al. 2014; Cerezo & García-García 2017, Cerezo et al. 2017). In the case of *O. maya* the studies are performed only with juveniles produced under controlled conditions (hatchery), preferably in ponds, and important progress has been made regarding feeding strategies and the development of nutritionally studied diets. In both species, there is already a basic formulated diet that can replace the fresh refrigerated diet that has been used traditionally.

The results for *E. megalocyathus* and *O. mimus* are based on experimental ongrowing of wild juveniles. Particularly, in *E. megalocyathus* there have been advances in ongrowing nutrition but the search remains for a diet that can replace the fresh diet (Gutiérrez et al, 2015, Martínez-Montaño et al. 2016). Since the use of formulated feeds is one of the critical steps to move towards sustainable aquaculture (FAO 2016), the ongrowing of these complex species is still at an experimental stage. The Mayan octopus in an excellent model to progress in the cultivation of these species for ongrowing or restocking. The production of juveniles for ongrowing requires a focus centered on the quality and quantity of the post-settled organisms for greater economic return, where the culture of the post-settled organisms is a tecnological constituent that must be added to the hatchery technology.

In Mexico, ongrowing cultures from laboratory-produced *O. maya* juveniles have been successfully developed by women's organizations, who are the wives of artisanal fishermen (Vidal et al. 2014). In Chile, artisanal fishermen who currently manage benthic resources under TURFs have shown to have a high capacity and interest in developing cultures as a way to make their subsistence production more predictable (Gajardo & Ther 2011; Salinas & Ther 2011), therefore, encouraging the cultivation of octopus or loco in these communities could be a potential success.

Currently it is not possible to provide the necessary production of juveniles in hatchery for ongrowing, the supply of juveniles from the fisheries is not predictable, and there is not yet a commercial feed rendering high performance (García García et al. 2014; Vidal et al. 2014). Besides, there is a risk for the sustainability of the natural populations of marine carnivorous mollusks when their recruits are used for ongrowing operations, making it a research priority to solve the bottlenecks that brake the production of juveniles of these complex marine species (with larval phase) under controlled hatchery conditions

Conclusions

The priorization of research to close the gaps in larviculture to achieve control and success in the juvenile production of the reviewed species must be linked with criteria for an intensive and sustainable production.

This work brings together the view of different specialists in the areas of larviculture, physiology, ecology, nutrition and animal health, on how to deal with the aquaculture farming of species with complex life cycles in a multidisciplinary way using as models the octopus and the muricid *C. concholepas*, with the aim of reducing the gap between the experimental culture and the industrial crop of species that are relevant for the diversification of aquaculture, particularly in Chile.

Research should be prioritized to standardize the production of early juveniles of octopus and *C. concholepas* from paralarvae and larvae cultures, respectively. This standardization must come primarily from the behavioral, physiological, nutritional and genomic knowledge of these species.

The current technology for producing juveniles from paralarvae or larvae is based on them being fed live prey that is either unsuitable or scarcely available, under non-optimized conditions of rearing. The reason behind this is the insufficient knowledge on the species-specific that does not allow sufficient survival of immature stages, in quantity and quality, to overcome the limited success of hatchery production for octopuses and *C. concholepas*.

The feeding and nutrition of octopus paralarvae requires the replacement or supplementation of live diets with inert diets (microencapsulated, among others), to achieve significant survival values. The production of *C. concholepas* juveniles not only faces a nutritional problem, it is also urgently necessary to improve the technological factors of larviculture that allow a correct physiological performance of the larvae. Therefore, although all species have difficulties reaching the final planktonic phase prior to juvenile, they differ in the type of bottleneck they face to reach it. For example *E. megalocyathus* and *C. concholepas* are characterized as species with the longest first stages of life compared to the other 3 species covered by this review. Out of all species, *E. megalocyathusis* is probably the only one with 5 months of embryonic development. Besides, all species except the Mayan octopus present difficulties in the survival of the early life stages.

Moreover, temperature is another factor that determines the growth and distribution of these species, a factor that is limiting especially during egg incubation and the period of immature planktonic organisms, larvae or paralarve. There are no studies on the physiology of larvae and early juveniles of loco associated with the effect of temperature and global warming, whereas in *E. megalocyathus, O. mimus* and *O. maya* this type of studies has already begun. Studies on these subjects are a matter of utmost urgency to the global warming scenario that could affect recruitment and survival of species.

Studies on reproductive physiology in octopus and *C. concholepas*, are currently insignificant if compared to this type of research on fish. Its relevance increases to an aspect of great interest when these values are crossed with the results of the state of genetic erosion of the species in wild communities. From a genetic point of view, there is relevant information on molecular markers and characterization of the natural populations of loco, while it is quite scarce on octopus.

The health status of these resources in wild populations and potential diseases due to captivity (or cultures in confined environments), have been further investigated in Europe for *O. vulgaris*, where it has been cultured for longer. The lack of health management and biosecurity information on native species of octopus and loco needs to be corrected promptly All these marine resources require the study of genomics associated with the innate immune system and the effect of nutrition and environment on gene expression, to understand in an integrated way the key processes for the development and growth of immature stages, reducing mortalities significantly and in the short term. Perhaps molecular biology is the most important tool to delovelop for the study of the larviculture of marine species with complex life cycle, due to the high quantity, quality and sensitivity of the information that can be produced from a small sample.

The requirements to develop standardized and cost effective juvenile production technology, whether for restocking or ongrowing, will allow to overcome the challenges of global warming and the overexploitation of wild stocks of these species.

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Table 1.- Challenges for massive production of juveniles under controlled conditions for reviewed species.

SPECIES (and distribution)	LIFE SPAN	REPRODUCTION STRATEGY	TYPE OF HATCHLING	FECUNDITY /HATCHING RATE	MAIN CHALLENGES FOR MASSIVE JUVENILE PRODUCTION	AUTHORS
O. vulgaris Cosmopolitan species of Mediterranean, and eastern, northeastern and southwestern Atlantic	1-1.5 years	Semelparous	Planktonic Hatchling of 3 mm TL	High fecundity (100.000-500.000 eggs/female) Hatching rate 20%	Food for paralarvae that can improve the survival to reach the total period of 47-52 days. Food for early juveniles Set-up of appropiate biotechnologies tools to understand the development of larvae	Villanueva et al 1997, Villanueva & Norman 2008, Hormiga et al. 2010, Vidal et al. 2010, Iglesias & Fuentes 2014, Garrido et al. 2017
O. maya Endemic species of Yucatán Peninsula (México)	2 years	Semelparous	Direct development Hatchling of 146±20 mg wet weight	Moderate fecundity (746±78 eggs/female) Hatching rate 92%	Food for early juveniles that can be produced at industrial level and can be stored and transported in a cheap form Set-up of appropiate biotechnologies tools to understand the development of larvae	Arreguín-Sánchez 1992, Rosas et al. 2014, Caamal-Monsreal et al. 2015
E. megalocyathus Endemic species of southern tip of South America (southern Chile to southern Argentina, including the Malvinas Islands and Burdwood Bank)	2,5 year	Semelparous	Planktonic Hatchling of 14.1±0.35 mm TL, 148.7±10.6 mg wet weight	Moderate fecundity (1.000-5.000 eggs/female) Hatching rate 15% ±8 38% ±3	Food for paralarvae that can improve the survival to reach the total period of 90-110 days. Food for early juveniles Reduction of cannibalism behaviour Set-up of appropiate biotechnologies tools to understand the development of larvae Production of juveniles for restocking ensuring maintenance of the genetic diversity	Uriarte et al. 2014, Uriarte & Farías 2014, Uriarte et al. unpublished results

O. mimus Endemic species of Southeast Pacific (northern Perú to central Chile)		Semelparous	Planktonic Hatchling of 2.0-2.42 mm TL	High fecundity (60.000-400.000 eggs/female). Hatching rate 75%	Standardized zootechnical conditions that allow the complete paralarvae development.	Warnke 1999, Castro el al. 2002, Zúñiga et al. 2014, Markaida et al. 2017
C. concholepas Endemic species of Southeast Pacific (central Peru to southern Chile, including the Juan Fernández archipelago)		Iteroparous	Planktonic Hatchling of 0.25 mm TL	High fecundity (180.000 – 2.400.000 eggs/female) Hatching rate 58.8%	Standardized zootechnical conditions that allow to obtain competent larvae at day 120. Food for early juveniles Set-up of appropiate biotechnologies tools to understand the development of larvae Production of juveniles for restocking ensuring maintenance of the genetic diversity	Ramorino 1975, Disalvo 1988, Inestrosa et al. 1990

Figure Legends

Figure 1. Total loco (*Concholepas concholepas*) fishery production in Chile and Peru. Data from FAO Fishstat 2016.

Figure 2. Octopus fishery in the main fishing countries for the species. Data from FAO Fishstat 2016. One to four species are clustered together

Figure 3. Total octopus fishery production of *Octopus mimus* (Chile and Peru) and *Enteroctopus megalocyathus* (Argentina and Chile). Data from FAO Fishstat 2016. One to two species are clustered together

Fig.1

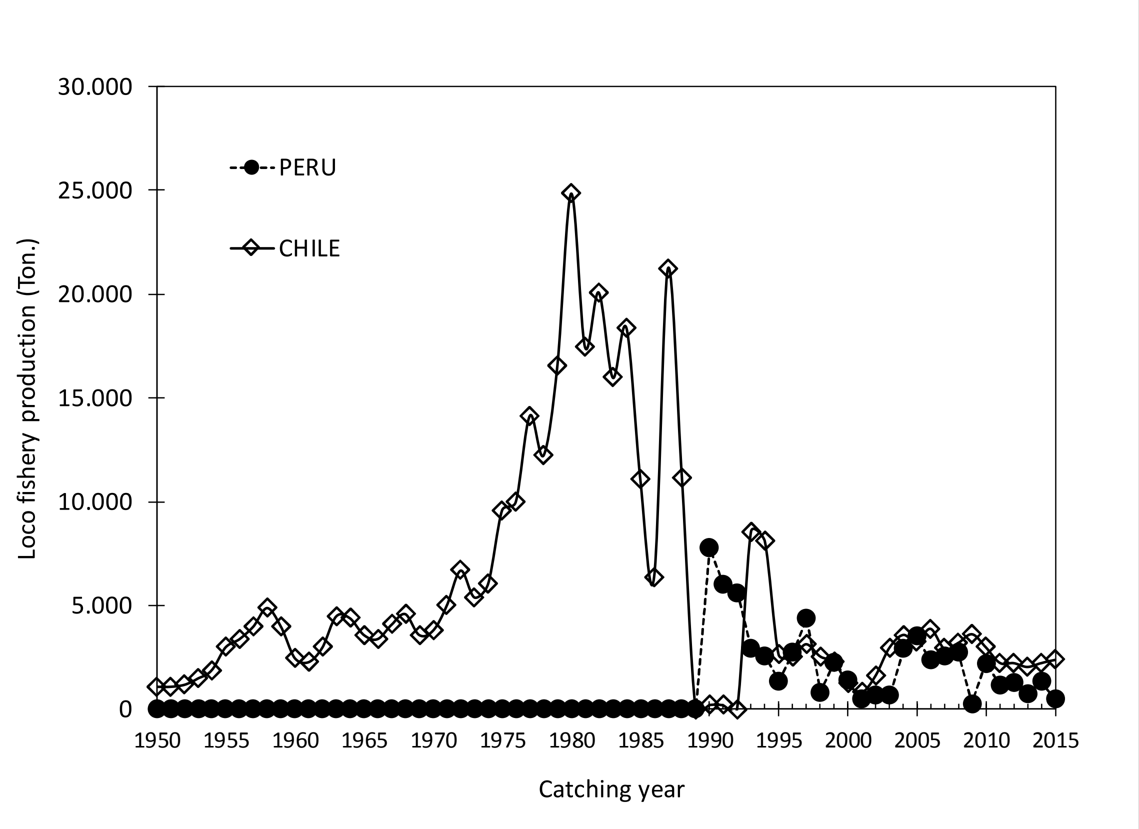

30.000
25.000
20.000
15.000
10.000
5.000
0
Loco fishery production (Ton.)
PERU
CHILE
1950
1955
1960
1965
1970
1975
1980
1985
1990
1995
2000
2005
2010
2015
Catching year

Fig.2.

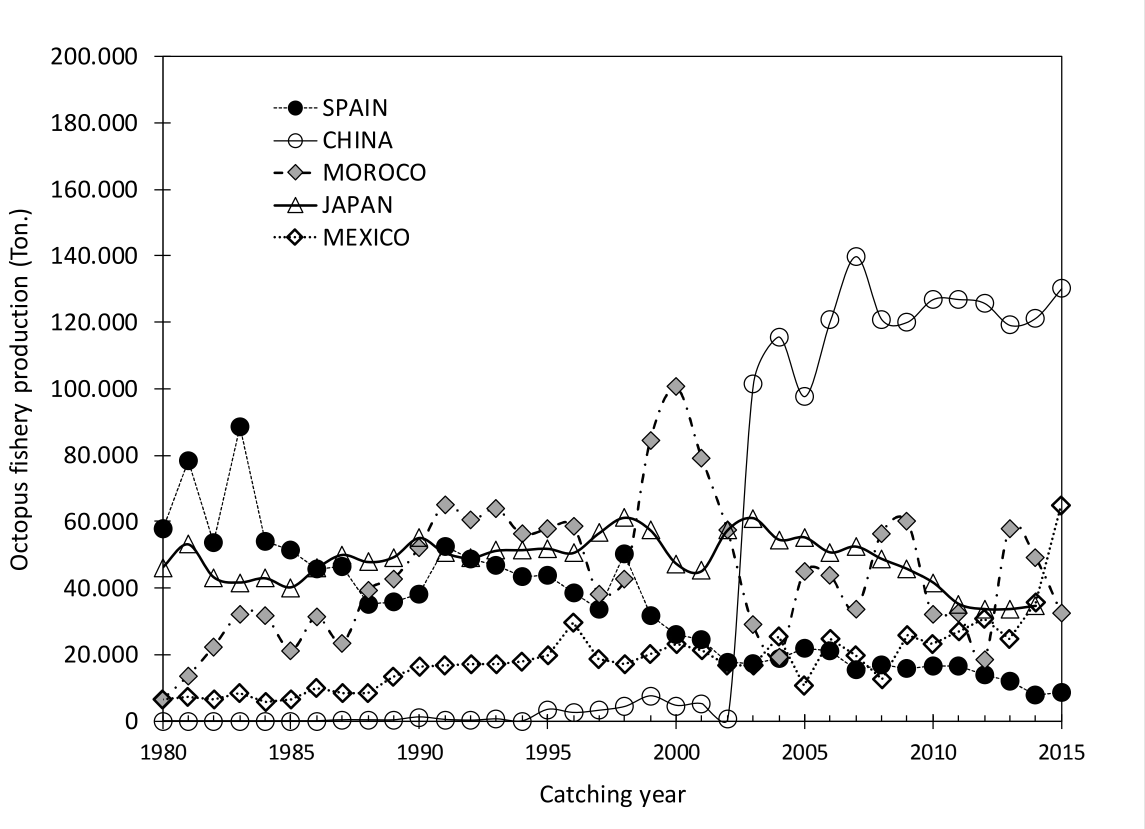
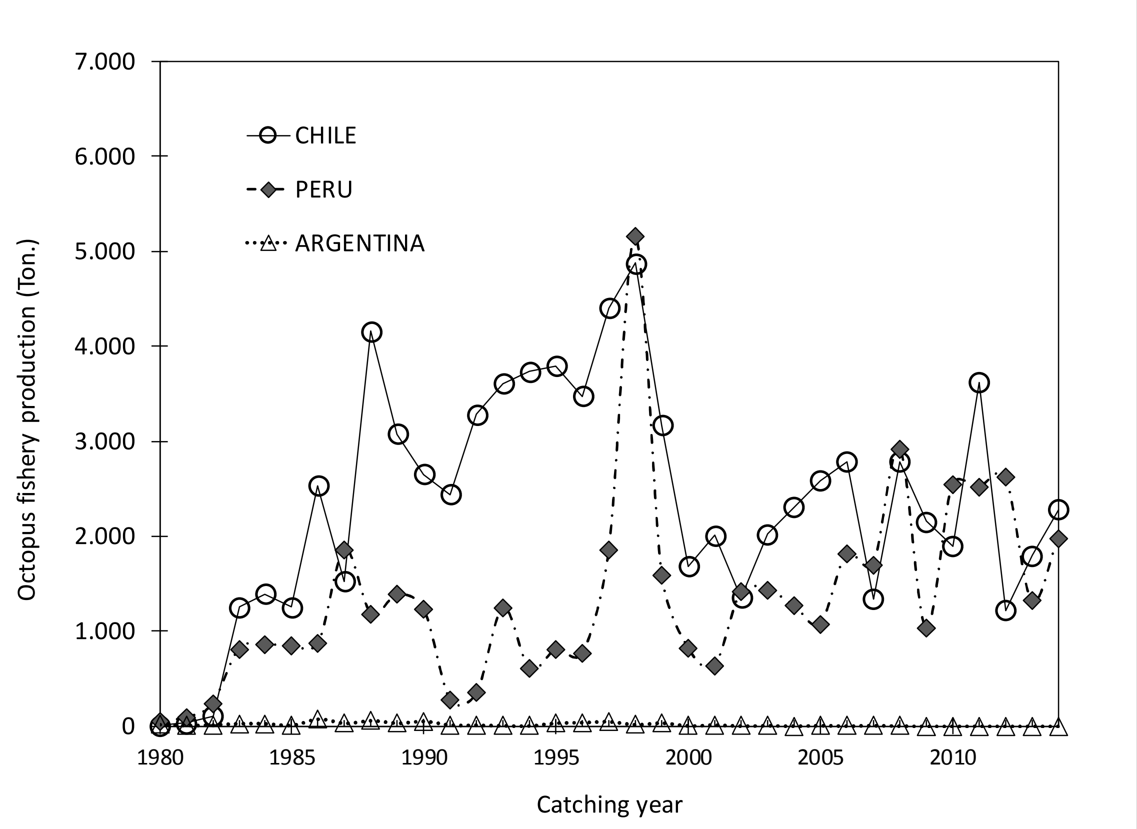

SPAIN
CHINA
MOROCO
JAPAN
MEXICO
Octopus fishery production (Ton.)
200.000
180.000
160.000
140.000
120.000
100.000
80.000
60.000
40.000
20.000
0
1980
1985
1990
1995
2000
2005
2010
2015
Catching year

Fig.3.


7.000
6.000
5.000
4.000
3.000
2.000
1.000
0
Octopus fishery production (Ton.)
CHILE
PERU
ARGENTINA
1980
1985
1990
1995
2000
2005
2010
Catching year