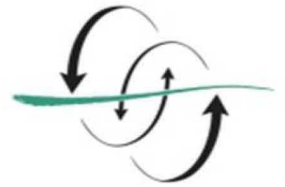




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Clownfish: commercial interest and culture



María Kristel Ortega García

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Tutores:

María Ascensión Viera Rodríguez

Lucía Molina Domínguez

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Nombre: **María Kristel**

Apellidos: **Ortega García**

Titulación que cursa: **Grado Ciencias del Mar**

Curso académico: 2013-2014

Tutor: **María Ascensión Viera Rodríguez**

Departamento de Biología de la Universidad de Las Palmas de Gran

Canaria Cotutor: **Lucía Molina Domínguez**

Investigadora asociada al IUSA

María Kristel Ortega García

María Ascensión Viera Rodríguez

Lucía Molina Domínguez

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1. Introduction

The term "ornamental fish " is a generic term describing to those aquatic organisms supported in an aquarium with intention of ornament, including fish, invertebrate as corals, crustaceans, mollusks, echinoderms, as well as alive rock (Panné & Luchini, 2008).

The trade of original organisms of the marine world is increasing, nevertheless, one of the controversies that has generated the trade of marine species is that unlike the ornamental fish of freshwater where more than 90 % is produced in farms, only 2 % of the fish and 1 % of the corals are obtained by means of technologies of culture in captivity, since in the main his origin is the capture of the natural way (near 98 % of more than 1.400 commercialized species) (Wabnitz et al., 2003).

1.1. History of the aquarium

The culture of aquatic species of ornament, it arose with the upbringing of fish, activity as old as our civilization. It is believed that the first ones in developing this interest were the Egyptians, who raised fish in reservoirs for mystical and ornamental reasons; whereas the Chinese during the Dynasty Sung perfected the culture of golden fish and tents (koi), being the first nation in realizing exports of fish of ornament to Japan in the year 1.500 (Vieth, 1998).

In the particular case of marine aquarium species, this activity had his beginnings in the middle of XIX century. Nevertheless, the commercialization and export of tropical marine fish for the trade of aquariums began in Sri Lanka in the decade of 1930, to very small scale. The trade expanded during the decade between an increasing number of countries, favoring that the quantity of permissions for the compilation of species destined for marine aquariums was increasing in exporting countries as Hawaii and The Philippines (Wood, 2001b). Later it turned into a multimillionaire industry when, in the decade of 1970, fisheries were established across the tropical Indian Pacific Ocean and the Oceans and the Atlantic Ocean. Nowadays, 45 countries replace the global markets with estimations of 14-30 million fish per year and a value of 20-32 million of euros (Bruckner, 2005).

1.2. Ornamental marine species of commercial importance

The industry of the marine aquariums depends fundamentally on the supply of ornamental species of vertebrates and invertebrates. Fish and crustaceans stand out among the marine organisms of high commercial importance, because his colouring attraction, exotic form and particular behavior. Nevertheless, the international market of marine aquarium species is dominated by the fish, with a volume of annual average production of approximately 20 millions of tropical fish (Cato & Brown, 2003; Wabnitz et al., 2003).

The majority of the saltwater fishes destined for his exhibition in aquariums, with the exception of the seahorses, needle fish and globe fish that they sell on the market as curiosities or articles for the home, or are used in the traditional medicine (Bruckner, 2005). The preference on the market is dominated by juvenile males of target species due to the fact that they present major color that the females and they survive more time that the adult organisms, besides which his transport turns out to be easier and economic. Of 1.471 species of fish commercialized in the world, the family Pomacentridae, he understands the species of major commercial appraisal, with approximately 350 species (Figure 1). The majority of these species are original of the tropical zone of the Indian Ocean and western Pacific Ocean (Reynoso et al., 2012).



Figure 1: The main commercial species of Pomacentridae family. Images taken from Google/Images.

The damselfish (Pomacentridae), clownfish (Pomacentridae) and angelfish (Pomacanthidae), represent approximately the half of the trade (Figure 1). Other species with relevancy on the international market there belong to the group of the fish surgeons (Acanthuridae), the wrasses (Labridae), gudgeons (Gobiidae) and butterfly fish (Chaetodontidae), which as a whole they constitute 33 % (Wabnitz et al., 2003; Bruckner, 2005). Inside these groups, ten species of major demand are: *Amphiprion ocellaris* (Pomacentridae); *Chromis viridis* (Pomacentridae); *Labroides dimidiatus* (Labridae); *Chrysiptera hemicyanea* (Pomacentridae); *Salarias fasciatus* (Blenniidae); *Chrysiptera cyanea* (Pomacentridae); *Paracanthurus hepatus* (Acanthuridae); *Synchiropus splendidus* (Callionymidae); *Pseudanthias squamipinnis*

(Serranidae); *Acanthurus leucosternon* (Acanthuridae), (Figure 2) that represent 36 % of all the fish commercialized (Wood, 2001b).

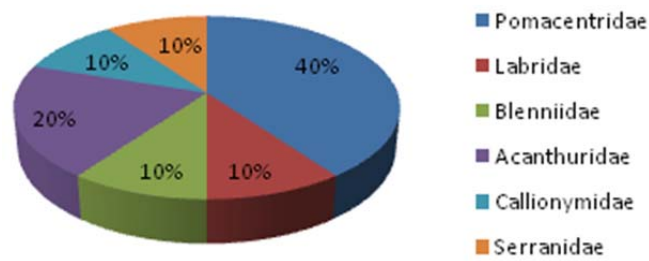


Figure 2: Shows the percentages of the most demanded families. © María Kristel Ortega García using data of Wood, 2001b.

From the reference analysis of I can conclude that this is due to the fact that the species of clownfish who belong to the family Pomacentridae, are one of the few ornamental species that have managed to be cultured obtaining increasing survival rates in the last years. Nevertheless, other species from other families are more difficult to cultivate up to now due to the lacking knowledge regarding its culture, consequently exporting countries are obliged to collect them from wild, in higher numbers. For this reason, the family Pomacentridae represents the major percentage inside the total, since the effort to obtain these species is very much minor, which facilitates that major quantities are exported.

1.3. Importance of Pomacentridae family

This family was the first marine ornamental fishes to be cultured, but considering their importance in the aquarium trade (it represents almost half of the traded species, a great amount of research is still being focused on breeding these fishes. This family includes damselfishes (*Chrysiptera spp.*) and clownfishes (*Amphiprion* and *Premnas spp.*), which are perhaps the most famous species in marine aquaria. Although the reproductive behavior of damselfishes has been described for some time, most available information is related to clownfishes (Molina & Segade, 2013). Some of the species of this family are commercially available, such as *Amphiprion ocellaris*, *A. melanopus*, *A. frenatus*, *A. percula*, and *Premnas biaculeatus*. This is probably the best known family among aquarists and is the most traded family because of their great strength, bright color and small size (Molina & Segade, 2013).

Clownfishes are the most longstanding and intensively cultured family of marine ornamentals and are the best ranked in marine aquarium trade. Also they were the first reef fish species bred successfully in captivity (Madhu et al., 2013).

1.3.1. International trade of marine ornamental species

The trends on the international market have demonstrated the expansion of the exports and imports of ornament species of. According to FAO (2007), from 1976 to 2004, the number of exporting countries extended from 28 to 146 and the importers grew from 32 to 120, of which 80 are those who supply the world market of ornamental marine fish (Gasparini et al., 2005). Nowadays, 85 % of the marine species that are commercialized comes from tropical and subtropical countries of the region Indo - Pacific, being Indonesia and The Philippines, the major exporters of the principal markets of the world, though The Maldives, Vietnam, Sri Lanka and Hawaii (USA) also supply an important number of organisms for marine aquariums (Bruckner, 2005). Another country that has acquired world relevancy in the last decade is Brazil, turning into one of the principal exporters of saltwater fishes (Gasparini et al., 2005).

In relation to the imports, The United States is the major importer, reaching near 80 % of the exports of hard corals and 50 % of the saltwater fishes, respectively (Wabnitz et al., 2003). Other important markets locate in the European Union (Germany, France, United Kingdom and the Netherlands) and the south east of Asia (especially Japan) that as a whole constitutes 26 % of the market of fish (Reynoso et al., 2012; Wood, 2001b).

On the international market, the marine species show prices very superior to those of fresh water, in spite of the fact that 90 % of the commercialized value is associated with species of freshwater and only 10 % with marine species (Reynoso et al., 2012). Even if exact information does not exist with regard to the value and the international trade of fish and invertebrates of ornament, it thinks that his import generates earnings for approximately 204 million of euros, of which from 20 to 32 million of euros correspond to ornamental marine fish (Bruckner, 2005).

1.3.2. Major exporters and importers countries

The Global Marine Aquarium Database (GMAD) was set up in 2000 as a collaborative project between the United Nations Environment Programme World Conservation Monitoring Centre and members of trade associations in exporting and importing countries. It compiles accurate quantitative information on the aquarium trade through centralizing and standardizing sales records provided by aquarium wholesalers. Relevant information from these records is then placed in the public domain. According to data held in GMAD a total of 1,471 species of fish are traded globally. Most of these species are associated with coral reefs although a relatively high number of species are associated with other habitats such as seagrass beds, mangroves and mudflats (Wabnitz, 2003).

According to data provided by exporters, the Philippines, Indonesia, the Solomon Islands, Sri Lanka, Australia, Fiji, the Maldives and Palau, together, supplied more than 98 per cent of the total number of fish exported between the years 1997 and 2002. (Figure 3, Left). GMAD trade records from importers for years 1997-2002 showed that the United States, the United Kingdom, the Netherlands, France and Germany were the most important countries of destination, comprising 99 per cent of all imports of marine ornamental fish. Exporters' data revealed Taiwan, Japan and Hong Kong to be important importing areas. (Figure 3, Right) (Wabnitz, 2003).

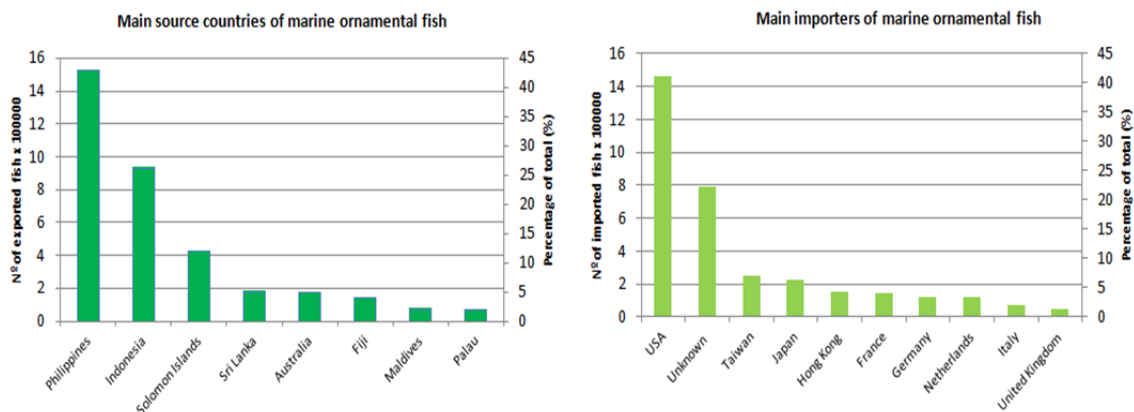


Figure 3: Graph showing the major exporters and importers of marine ornamental fish countries. © María Kristel Ortega García using data of Wabnitz, 2003.

Looking at the most commonly traded families, species of Pomacentridae dominate, accounting for 43 per cent of all fish traded. For the years 1997-2002, the blue-green damselfish, the anemonefish, the whitetail dascyllus, the sapphire devil and the threespot dascyllus are the most commonly globally traded species. The top ten

species together account for 36 per cent of all fish traded from 1997 to 2002, according to data provided by importers (Wabnitz, 2003).

1.3.3 Wild caught animals and the impact on the ecosystem

As with any use of natural resources, there are a number of problems and issues of concern relating to the collection of marine ornamental species. The potential environmental and biological impacts of these fisheries have been recognized for many years and include: overharvesting of fish and invertebrates, changes to the ecology of the reef due to collecting, degradation of coral reefs due to physical damage inflicted by collectors and their gear, degradation of reef from use of cyanide and other poisons, loss of biodiversity due to these factors (Wood, 2001a).

The 90 % of the marine species commercialized nowadays is captured in the reefs of coral or the adjacent areas and though the reefs cover less than 0,25 % of the marine environment, is considered to be ' the jungle of the sea ' due to his high biodiversity. They are the biological more productive ecosystems of the world and receive to more than 4.000 species of fish. In addition, a great number of species use these ecosystems as area of reproduction or baby's zone (Molina and Segade, 2013). The 20 % of the reefs of coral of the world has been destroyed by efficiency and does not show immediate perspectives of recovery (Veron et al., 2009).

The threats that concern the reefs of coral are divers. Between them there are the ornamental fishing, the jeweler's shop and curiosities for the trade, material of construction, as well as traditional medicines and pharmaceutical products. On the other hand, the destructive, such technologies of fishing like the use of dynamite or cyanide of sodium, often they are in use for stunning and catching ornamental species, producing the severe and long term hurt not only to the target species, but also to the surrounding habitat. The effect of these harmful chemists for the habitat continues to be unknown stranger, though other technologies of compilation like the dynamite and the explosives affect too many more species that can turn damaged, wounded or murdered. All these methods produce serious hurts in the populations of organisms and also the destruction of big areas of the reef of coral (Molina and Segade, 2013).

Whereas it is required a great diversity of species for the trade of the aquarium, a significant part of the trade tends to be characterized by an extremely selective fishing and that centers on a few species of high commercial value (Wabnitz et al.,

2003). This there reduces clearly the population of these species in concretely and makes diminish the diversity of these concrete zones where there is realized this type of selective fishing. Another topic of worry is the selective withdrawal of the juvenile ones and the males due to his distinctive coloration, which it generates, unbalanced populations, concerning the recruitment and the reproduction (Wabnitz et al., 2003).

In addition, the overexploitation of organisms known as cleaners, such as gudgeons (*Gobiosoma spp.*) and maidens (*Labroides spp.*), it can concern also negatively the ecology of the reef fish, especially for the members of a symbiotic relation. These species play an important role in the reef health, since they take charge of the elimination of parasites and other materials, as the mucus or the dead tissues (Molina and Segade, 2013).

In general, the species of fish with narrow geographical ranges can be the most vulnerable to the exploitation, but also depending on the abundance. Some biological, such factors as the behavior, the care parental, the fecundity, as well as the speed and the frequency of the recruitment have a significant impact in the recovery of species.

1.4. Family and genus characteristics

Amphiprioninae is a subfamily of saltwater fishes of the family Pomacentridae, known as clownfishes or anemone fish. It belongs to the class of the Actinopterygii since they are fish with removed fins and perciformes for his form of perch. They are tropical fish (6°S - 26°S, 141°E - 155°E) that are associated with the reefs of coral. They are not migratory fish and are in the habit of living between 1 and 15 meters of depth. As for his morphology, it possesses between 9 and 10 backbones, 14-17 soft dorsal radiuses, 2 anal thorns and between 11 and 13 soft anal radiuses (Fishbase, 2014).

1.4.1. Taxonomy

Clownfish belong to the widely diverse and well distributed family Pomacentridae (damsel-fishes). There are 27 known clownfish species. They are distinguished and taxonomically separated from other damselfish by their dependence on anemones for protection (Hoff, 1996).

1.4.2. Biology

Anemonefish, as their name defines, live in a mutualistic relationship with anemones. Primary benefits to clownfish from anemone association are protection of the pair, their nests and a portion of their progeny from predation. Anemones are known to derive their coloration and substantial amount of their nutrition directly from commensal microalgae called zooxanthellae which live within their surface tissues. Since nutrients are very limited in tropical reef situations, it is possible that the urea and fecal matter excreted by the pair of clownfish serves as a basic nutrient source which in turn enhances growth and survival of the zooxanthellae which in turn provide nutrients to the anemone (Hoff, 1996).

The clownfish can survive the stinging cells of the tentacles of the anemones since they develop a mucous cap special day pupil. This cap seems to be three or four times thicker in comparison with other species of fish that do not use anemones. The fish are covered with a small quantity of mucus of the anemone when the contact takes place with his host (Gail, 1991). Nevertheless, in captivity, the need and dependence of the anemones it diminishes since the possibility of the depredation diminishes. The functions of the anemone are replaced by a worthy housing that favors spawning and the isolation of other fish that interrupt the tranquility of the breeding pair (Hoff, 1996).

In the wild, selection of specific anemone species may simply be based on location of specific anemones in relationship to current, light, reef habitat and specific food sources. (Hoff, 1996).

1.4.3. Distribution

The clownfish of the genus *Amphiprion* and *Molva* they are along the region of the western Indo - Pacific Ocean (Figure 4). The center of the abundance is the region Indo - Australian in the archipelago of The Philippines, which area contains 10 known species. The genus *Amphiprion* could have penetrated in the majority of the Islands of the tropical western Pacific that are numerous and generally they are not isolated of the neighboring islands. Nevertheless, where there are big extensions of opened waters, as well as between the Islands of the Marshall and Hawaii or between the islands of Easter, *Amphiprion* could not have penetrated (Hoff, 1996).



Figure 4: Distribution of clownfish species. Images taken from Fishbase

In addition, the availability of the anemones they concern the distribution of the clownfish and the such hydrographic factors as deep and cold waters of outcrop close to shallow lagoons hot, the rapid adjacent currents the coasts that sweep the larvae towards the deep waters or the sedimentation and run-off of sweet water of the rivers, can be factors of isolation and regulators of the distribution. Therefore, Amphiprion distribution can divide in three areas: (1) those who are widely distributed (*Amphiprion clarkia* presents the widest distribution); (2) the species that are limited by the regional distribution and (3) those confined in specific islands or groups of islands (Hoff, 1996).

2. Culture of clownfish

To be able to cultivate the clownfish is necessary to know all the aspects related to the sexuality, the feeding habits and the maintenance of this species in captivity, and that are described detailed following:

2.1. Sex changes

Anemonefishes are well known that in their coral reef habitat live in social groups usually composed of one breeding pair and a varying number of non breeders. The female is the largest and dominant member of a social group, displaying dominant behavior toward other group members. The second ranked individual is the breeding male, and others remain as non reproductive individuals with ambisexual gonads because adults inhibit the growth of juveniles by social interactions (Iwata & Manbo,

2013). All anemonefish are protandric hermaphrodite, starting life as a male and later changing to a female and most anemonefish species form long term monogamous pairs. The male–female bond only breaks when one member of the pair is lost. In most cases, the female is larger making it more vulnerable to predation. If the female die, the surviving male if the pair retains the ability to change to a female and one of the associated juveniles assumes the new male position (Yasir & Qin, 2007).

The protandry and social system of anemonefishes have been regarded as adaptations to the extreme difficulty of moving between hosts due to the low population density of sea anemones and related predation pressures in subtropical to tropical waters (Hattori, 1991).

During early development, a fish larva is, phenotypically, neither male nor female in that it does not possess ovaries, testes, or other characteristics associated with the reproductive systems. Instead, an embryo possesses embryological precursors of ovaries and testes, and at this stage an embryo is “totipotent” because it could develop into either a male or female. At a specific time during embryological development, a chemical signal originates from a gene or set of genes and this signal “informs” the totipotent tissue which way to develop. It is during this stage that breeders often attempt to hormonally alter populations into mostly male or mostly female fish depending on what is desired (Hoff, 1996). However, in anemone fish, ovarian differentiation takes precedence over testicular development, and differentiation of testicular tissues conforms after five months of ovarian development (Miura et al., 2003).

2.2. Spawning and hatching

From the studies Frank H. Hoff published in 1996 he described the different behaviors that show the clownfish before, during and after the process of spawning and that they explain following:

Spawning within the central regions of clownfish concentrations occurs about once a month and a higher percentage of spawns occurs within six days prior to and after the full moon. The female took the lead role and they normally spawned in the early afternoon. Spawning and hatching occurs during the period of the full moon because lunar periodicity may facilitate nocturnal egg care. Lunar light might also benefit newly hatched larvae since they are photopositive. Low light may simulate

larva to swim up into the water column immediately after hatch and allow greater dispersal of hatchlings since surface because that the tidal currents are greater during the period of the full moon.

Often the female takes the initial lead role in forming a new pair and in spawning. She will clean several hard surface areas and often pushes the male in his side or belly as if teaching him what to do. At times she appears aggravated, frustrated and nervous, often shaking her head. Eventually she may actually push the male toward the spawning substrate. Head shaking, standing on their heads, nipping and chasing are common prior to spawning, especially in new pair. In conjunction with this display, excessive digging in the bottom substrate or cleaning of many areas usually occurs.

As a pair matures, both become active in site selection and cleaning which occurs 3 to 5 days before spawning. As female become sexually mature their physical appearance changes. They begin to display a “squaring” of the abdominal area (Figure 5) which is actually internal maturing of eggs into a pre-spawning state within the ovary. At this stage they will often dig holes in the gravel below and around the spawning substrate.

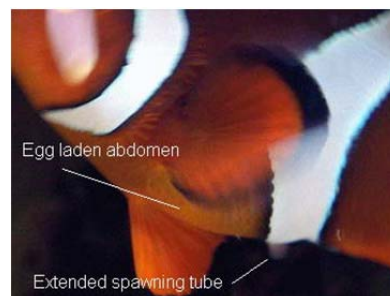


Figure 5: Physical appearance changes: “squaring” of the abdominal area. Images taken from Google/Images and modified

Females laid capsule-shaped eggs on the cleaned substratum in almost circular or oval patches and subsequently the male fertilized the eggs. The spawning activity lasted from an hour to an hour and forty five minutes. The eggs are adhesive and are covered with a transparent chorion and a narrow perivitelline space. The eggs measured 2.0–2.1 mm in length and 0.9–1.0 mm in width. Approximately 300–400 eggs were spawned at a time. The number of eggs gradually increased in subsequent spawning. One end of the egg capsule identified as the animal pole contained some gelatinous substance to adhere itself to the substratum.

Clownfish normally spawn in mid-afternoon, before dark. Once spawning commences, female press their body towards the substrate, quiver slightly and slowly move in a rowing fashion using her pectoral fins. She moves in a circular path depositing a continuous spiral of eggs from the center outward (approximately 60 eggs per square centimeter). The male swims behind the female, releasing sperm over the newly deposited eggs. Often males go back over areas and release more sperm after the female has moved aside.

After spawning, the male starts to assume a more dominant role because parental care of nest is mainly the function of males. After spawning, both parents usually attend the nest until larvae hatch. The fanning behavior of the parent fish brings oxygen to the nest and removes dead eggs (Green & McCormick, 2004). Fanning eggs is frequent on the day after spawning and diminishes considerably about midway in the incubation period. On the day of hatch, fanning increases again. Fanning is usually accomplished by utilizing pectoral fins and occasionally caudal fins. "Mouthing" of eggs by both fish is common. They literally place their mouths around the eggs. This is considered a cleaning process of "mouthing" fish secret and antibacterial / fungal slime on the eggs (Hoff, 1996).

Dominant pairs are more likely to lay eggs around lunar day 19 than at other times, they do not lay eggs if they already have eggs, and they are more likely to lay a first clutch than they are to lay a second or third clutch in a given lunar month. They indicate that the probability of laying eggs fluctuates with lunar month (Buston & Elith, 2011).

2.3. Egg development

The embryonic development of fish refers to the period during which the developing individual is entirely dependent on nutrients provided by the yolk from the egg. The embryonic period begins upon the fertilization, and can be divided into two phases. The first is the egg cleavage phase, which is the interval between the first cell division and appearance of recognizable predecessors of the organ system, especially the neural plate. The second is the embryo phase during which the embryo becomes recognizable as a vertebrate (Ghosh et al., 2012).

The newly laid egg is orange in color, elliptical in shape and slightly curved around the middle part of the yolk. The chorion is transparent and the egg could be seen through the shell (Figure 6, Day 0) (Yasir & Qin, 2007).

Development is quick. At approximately 24 hours the early nurula (earliest stage of obvious body form) is evident. At 48 hours; eye, heart, brain and spinal cord are present and the yolk is slightly reduced and darker orange. The third night, the embryo reverses its position, by aligning its head at the unattached end of the egg. By the fourth day, darkly pigmented eyes and body parts are evident. By day five, pectoral fins and dorsal and ventral fin folds are apparent, also major blood vessels and circulation are clearly evident. By the sixth day semicircular canals, mouth and well developed eyes consisting of blackened iris and silver covering are apparent. Operculum flaps are barely discernible. Day seven is similar to day six except more refined, vestiges of gill rakers and developed gill flaps are evident, also yolk is considerably smaller (Figure 6) (Hoff, 1996).

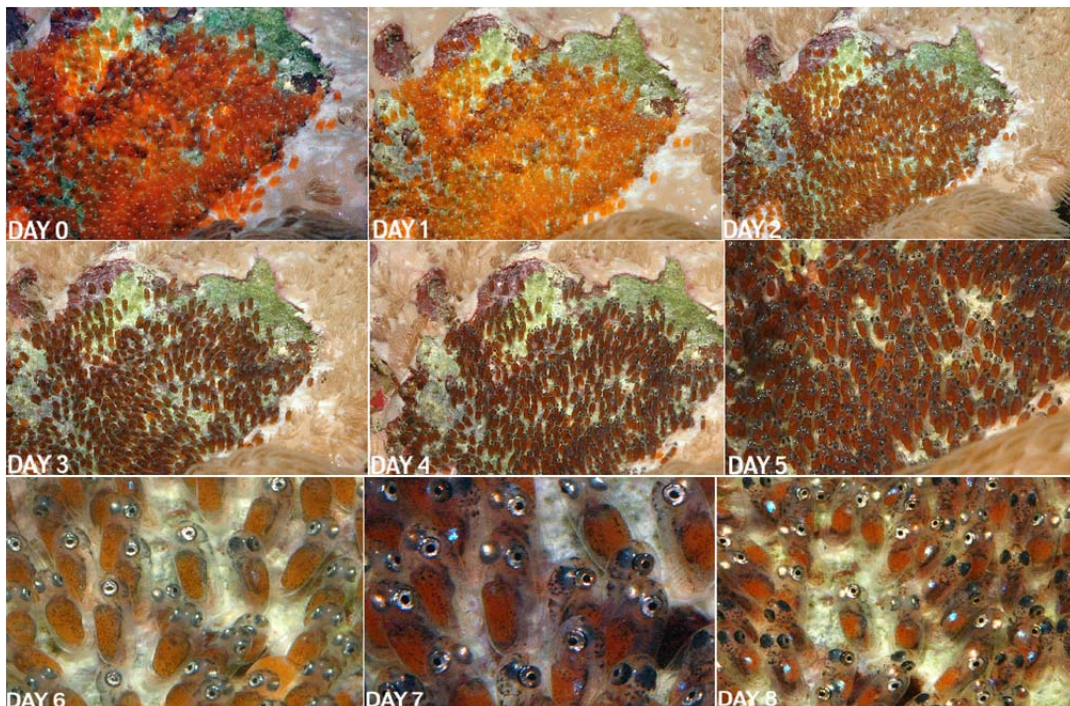


Figure 6: Egg development since spawning until hatching. Images taken from Reefsmagazine

During early development the larva's head is located at the attached end of the eggs. When about to hatch, they rotate inside the eggs so that their head is at the unattached tip. At hatch, they push forward with their tails, breaking the unattached tip of the egg (Dhaneesh et al., 2009). The length of the newly hatched larvae range

from 2.5 to 3.0 mm approximately and the larvae metamorphose into juveniles 12-15 days post-hatching (Figure 7) (Sahandi, 2011).



Figure 7: Larvae development from hatching to the juvenile stage. Images taken from Reefsmagazine

Some determinants for successful reproduction effects are described by Buston & Elith, publicized in 2011. These studies indicate that change in male length is negatively related, and year clutch is positively related to the probability of laying eggs and the number of eggs laid per clutch. These effects, taken together, are the primary determinants of the total number of eggs hatched by pairs. Also, changes in female mass and initial female standard length are both positively related to the number of eggs laid per clutch. These effects, taken together, can be considered the secondary determinants of the total number of eggs hatched by pairs.

From the study of articles, I believe that on having increased the size of the male, a major energetic expense takes place in his own maintenance and that therefore, a compensation effect takes place and that way, the energy used in the reproduction meets reduced unleashing a minor quantity of satisfactorily hatched eggs. In case of the female, an increase in her size and mass generates an increase of his thoracic cavity, which makes possible a major production of eggs and therefore, a major index of its satisfactory appearance.

2.4. Feeding habits

Anemone fish are classified as omnivorous, opportunistic feeders. This means they consume a wide variety of food items consisting of plant and animal matter and do not travel long distances to pursue their food. Feeding is generally confined to the surface area and water column immediate to their host anemone. Tidal flow and wave action supply bits of floating debris, mostly algae, into their water column. Most feeding ventures are along bottom substrates and do not expand beyond three to five meters from the host anemone (Hoff, 1996).

Hoff (1996) examined the stomach contents of 74 specimens from Eniwetok. By volume, 91.8% of their food consisted of an average of 34.8% copepods, 33% algae,

9.7% worms, 6.4% crustacean fragments, 5.2% tunicates and 2.7% amphipods. The remaining 8.2% consisted a wide range of food items including: isopods, barnacle nauplii, Amphiprion eggs, shrimps, crustacean larvae, barnacle appendages, unidentified eggs, crabs, nematodes, ostracods and polychaetes. It is important to note that copepods and macroalgae constituted 67.8% of their diet. But it is important to remember that feeding preferences in clownfish is probably forced upon them by their tendency to remain isolated and somewhat sedentary.

Clownfish larvae and juveniles have been reared successfully on highly integrated and diverse feeds such as rotifers, small particulate dry feed, small Artemia, krill meal, followed by larger dry feed particles and mixtures of natural feeds (e.g., chicken heart, fish roe, shrimp tails) (Gordon et al., 2000). When the larvae starting swimming they feed on rotifers. Rotifer feed would be used for a period 12-20 days. From the 5th day Artemia nauplii were used besides rotifer feed. Whenever possible, enrichment of rotifer or Artemia nauplii with Omega 3 and probiotics would be effective. This enrichment would improve the digestive tract bioprocesses and consequently the larvae survival and health (Sahandi, 2011).

Under my personal experience, the feed administration of the clownfishes in captivity is carried out using fish feed elaborated by different natural ingredients adding vitamins, minerals and pigments, and that then crimping to obtain pellets of adequate size according to fish size. In turn, four different fishfeed are used, one of them commercial and the other three formulated and elaborated in our laboratory. In these handmade diets different proportions of the ingredients were used, changing this way the proportions of proteins and lipids principally. This will make possible to know which is the most adequate food for this species after comparing values of growth, in weight and size, coloration and rate of reproduction.

2.5. Experimental work

During my training period I can see of firsthand how to carry out maintenance and relevant care to a number of breeding pairs of clownfish (Figure 8). The experiment used 24 breeding pairs of the species *Amphiprion ocellaris* (Cuvier, 1830) feeding on different fishfeed during the spawning period. The main aim was to know which diet was most adequate for this species, to study the period of pre - spawning, spawning and post - spawning and finally to study the development of the eggs and the larvae survival.



Figure 8: Experimental aquarium showing one of the breeding pairs with flowerpot used to collect the eggs. © María Kristel Ortega García.

2.5.1. Installation

The installation consists of two racks of aquariums. The system of support of life of every rack is integrated (biological, mechanical and ultraviolet filter) and it includes a climate for the regulation of the temperature. In total the installation presents:

- 2 racks of glass aquariums with 12 unit of 30 l and 3 unit of 90 l .
- 6 unit of 90 l aquaria (3 units for rack) used as system of mechanical and biological filtration.
- System of ultraviolet filtration integrated to the system of pipelines that allows the seawater inlet.

2.5.2 Maintenance

The circuit is closed, water inlet pass through an ultraviolet system used for water sterilization. It is necessary to verify the water level of the system daily since always the level of the water must be above or in the adequate, if not water must be refilled until the adequate level is reached.

Closed systems utilize water exchange but it is usually 20% per month. A distinct advantage of semi-open or closed systems is water stability. Water stability and balance is necessary within all aquatic bodies to keep animals growing, sexually mature and healthy. Recirculating systems offer this security and balance (Hoff, 1996) when adequate filtration systems were used.

The support life system is the most important part of any aquarium. It is the system of maintenance of life and must be considered to the beginning of the assembly of the installation to select the system most adapted to the type of fish that is going to be cultivated (Sandford, 1994). Maintenance of water quality is the most critical factor for clownfishes under controlled conditions. As a measure for this, the sea water need to be filtered through a series of sand filters before being taken to tanks (Madhu et al., 2013).

In our case, the filtration system is composed by a system of biological filter across bioballs that providing surfaces for the installation of the beneficial bacteria that carried the nitrogen cycle, a system of physical filter based on of sponges that allow to retain particles in suspension according to their size and a skimmer that eliminates the present ammonium in the water and that is toxic for the aquatic organisms. In our facilities these parameters are automatic controlled by means of a *Powerbox* that registers the data in PC.

The cycle of the nitrogen develops in anybody of water. During this cycle, the products of waste of the aquatic organisms, which are toxic for the aquatic life, turn into innocuous nitrates that then are absorbed by the plants or eliminated during the regular changes of water. In the closed ecosystems of the aquarium also this cycle exists and therefore, is needed of a skimmer to keep the levels of ammonia under control. Therefore, for the correct functioning of the skimmer the periodic cleanliness is necessary, specially of the interior pipe of the glass of skimmed off, to prevent that the air bubbles of the foam that ascend do not exploit on having hit against the residues deposited in the walls of this one (Sandford, 1994).

As for other maintenances, the cleaning is necessary using freshwater for the metallic elements, systems of pipelines and glass every week, since the high evaporation of the room produces an accumulation of great quantity of salt in these zones that it must be withdrawn to avoid the corrosion and the deterioration of the structures. In addition, the daily siphoned of the aquariums is necessary to avoid the

accumulation of excrements and remaining food in the bottom and in this way diminish the quantity of the ammonia in the system.

2.5.2. Parameters

Oxygen content is the key to the efficiency of biological filtration. The overall rate of oxygen consumption for a system is the sum of the respiration rate of the fish, oxygen demand of the bacteria breaking down organic wastes and uneaten food, and the oxygen demand of nitrifying bacteria in the filter (Hoff, 1996). In addition, salinity of the culture environment is an important abiotic factor that influences marine fish eggs and larval physiology and has a direct effect on their development, growth and survival. By influencing the energy requirement for osmoregulation, salinity directly affects the efficiency of yolk utilization, larval growth and survival (Dhaneesh, 2012).

For these reasons, the most important environmental parameters such as temperature (26 to 29°C), salinity (33 to 35 ppt), dissolved oxygen (4.6 to 6.2 ml/L) and pH (8.1 to 8.4) are to be maintained in all tanks, and also the water must be in movement to assure the good quality of the water (Fernando et al., 2006; Madhu et al., 2013).

3. Conclusions

- The culture of ornamental species constitutes a very old activity that in the 19th century extended to marine ornamental species confined in aquariums. The commercialization of these species had his beginning in the Indo - Pacific Ocean and The Atlantic Ocean that was consolidated as export zones. Hereby, there are located in these areas the principal exporting countries of the world.
- The 90 % of the commercialized species is captured of the marine environment, and due to the selective capture of especially of high commercial value species, a great loss of diversity takes place in the zones of exploitation. In addition, collateral hurts produce to themselves to other species or adjacent ecosystems due to the technologies of fishing, which brings with it serious consequences to the marine environment.

- On the market, the principal marine commercialized species belong to the family Pomacentridae being the damsels and the clownfishes they are the most out-standing species. This is owed principally to that there are of few ornamental species that have managed to be cultivated in captivity by satisfactory results, whereas of other species there are not known many aspects of his culture.
- The clownfish belongs to the family Pomacentridae and 27 species are known till now. Due to his symbiotic relationship with the anemones, they have a geographical distribution concentrated in the region Indo - pacific western. Of such that, his dependence to the anemones does that his distribution is tied to the geographical distribution of these, which it is limited by diverse hydrographic factors.
- The fact of being protandric species assures the continuity of the species after adverse situations as the death of the female. To live in small groups favors the protection of predators, though also the violent behavior is promoted between individuals of different hierarchic ranges.
- They present previous behaviors since it is the courtship and the morphologic changes, which make possible to feel the period of spawning. There has been verified that preferably they put his eggs to the afternoon and that the suitable conditions give themselves between them 26-29°C of temperature, 33-35 ppm of salinity, 4,6-6,2 of dissolved oxygen and 8,1-8,4 of pH.
- The care of the eggs is carried of both progenitors and they take charge of the aeration of the eggs and elimination of those who have not survived. Finally, in approximately 12-15 days after spawning, the metamorphosis takes place to juvenile organisms. A negative relation was observed between the size of the male and the eggs hatched and a positive relation with the female. Hereby, it comes near to the conclusion from which to major size of the male, major energy for his corporal maintenance and minor energy for the reproduction, whereas in the female to major size of same, major quantity of eggs in his thoracic cavity, and therefore, major possibilities of success.
- They are considered to be omnivorous organisms that are fed on great variety of wild food. For this motive, the most efficient diets for the culture of

clownfish are elaborated by natural ingredients trying to emulate the composition of their diets in natural conditions.

- About the experimental work, there were optimized the values of the suitable parameters for the reproduction, as well as the inlet and outlet system, ratifying the idea of the closed systems as the best option to support the stability of the parameters that make possible the reproduction under cultured conditions. Besides, different diets made experimentally to establish the most suitable set for quality, as well as survival, growth and coloration adequate specimens were tested.

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