Gramma loreto. About its reproduction

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Peculiarities of the species

Gramma loreto (Poey, 1868) is one of those species that immediately captures the attention of the aquarist, both for its attractive aesthetics and for some of its behavioral patterns. The males build "nests" inside their shelters with pieces of algae or pieces of substrate. They have the habit of swimming in an inverted position on the walls and ceilings of the hollows. Males with border territories measure their strength by grasping each other's jaws. In nature, they feed on pelagic and benthic planktonic organisms, mainly crustaceans, but also polychaetes, nematodes and algae. (Báez-Hidalgo,2002) [1]. Occasionally, the species also feeds on parasitic organisms. Eibl-Eibesfeldt in 1955 observed *G. loreto* " pecking" the bodies of other fish, apparently feeding on ectoparasites. Böhlke and Randall (1963)[2] found that the stomach of four specimens contained parasitic copepods. The species' literature considers its bathymetric distribution to range from 1 to 50 meters, but it has been located at 65 m depth (Mooi y Gill, 2002).

G. loreto. Couple in a hollow. Rep. Dominicana

G. loreto. Juveniles swimming in the upside-down position. Cuba

G. loreto. Group feeding on vertical wall. Puerto Rico

G. loreto is born with bisexual gonadal tissues, although once the sex of a given specimen is determined, only the gonads corresponding to a male or female specimen mature. (Asoh K et al, 1997)[3]. It is a gonochoric reproductive species, with the sexes separated from the juvenile stage and with no reversibility of sex under any circumstances (such as the isolation of an all-female group). *G.* loreto is socially organized in hierarchically structured groups, led by a dominant male. On average, these groups consist of 1-2 males, 2-9 females, and a variable number of juveniles. (Asoh, 1992)[4].

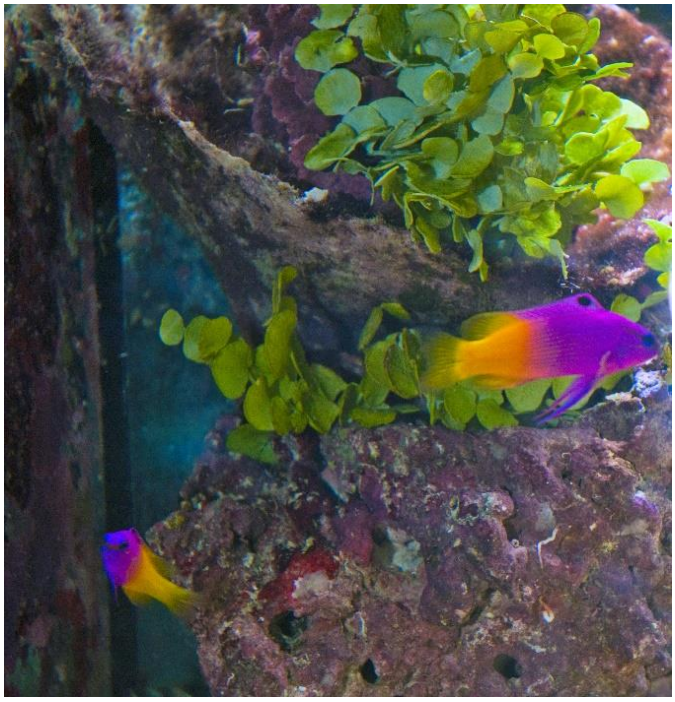
G. loreto is included in the genus Gramma[5] together with four other species: *G. brasiliensis, G. dejongi*, *G. melacara*, *G. linki. G. loreto* and *G. brasiliensis* share a very similar appearance, but among other differences, the shape of the dark band (yellow-greenish) that crosses the eye diagonally identifies G. loreto. On the other hand, *G. loreto* and *G. dejongi* share almost identical genetics with a lack of divergence in mitochondrial DNA sequence. (Victor, B.C. and J.E. Randall. 2010)[6]**.** The genus *Gramma* ("fairy basslets") together with the genus *Lipogramma* constitute the family *Grammatidae*, which is closely related to the "groupers" (*Serranidae*).

G. loreto. Couple during courtship. Males are usually larger than females and have more developed pelvic fins.

An easy and rewarding species to maintain

The good reputation of this species among aquarists is well deserved and certainly *G. loreto* is an excellent choice for a marine aquarium and especially for a marine reef aquarium. When I have had the opportunity to observe and photograph this species in the wild (mainly in Cuba, P. Rico and the Dominican Republic), I have generally seen small groups (according to my dive logbook, a minimum of two and a maximum of 16 specimens), and although each colony has its peculiarities, in general the structure described in the previous section can be seen, with the presence of a dominant male that stands out for its size and that you can barely see for a few seconds because as you approach the group, it quickly disappears into the crevices and hollows of the vertical portion of the rocky or coral littoral occupied by the rest of the specimens, mostly adult females and juveniles.

These and other characteristics of their biology are not difficult to extrapolate to an aquarium, to which *G. loreto* adapts easily. The social scheme observed in nature is feasible to reproduce in aquariums of more than 600 liters with adequate environmental enrichment: sufficient rocky and/or coral relief, simulating vertical walls with overhangs and abundant shelters (a minimum of two per specimen). In such an environment, the presence of other compatible species that do not pose an oppressive competition for space and/or food is an option that in my experience has not hindered the reproduction of *G. loreto*.



G. loreto. The rocky walls full of shelters are the optimal environmental enrichment for this species.

Another relevant requirement is to provide the aquariums with lid-grids, since they have the habit in high-stress situations (sudden switching on of ambient lights, rapid approach to the front, etc.) of jumping out of the tank.

Their feeding is not a problem, since they accept a wide range of live and frozen foods and even flake food. I personally like to prepare a porridge with fresh products from the fish market (shrimp, mussels, squid rings, a piece of some oily fish, macroalgae and vitamins A and E). Twice a week, they get an extra dose of lobster eggs.

It is a species that, once settled, is quite resistant to the most common diseases. If due to an accidental and prolonged drop in temperature they get sick, they usually respond very well to medication and hyposaline baths, with very fast recovery times.

Relevant aspects related to its reproduction

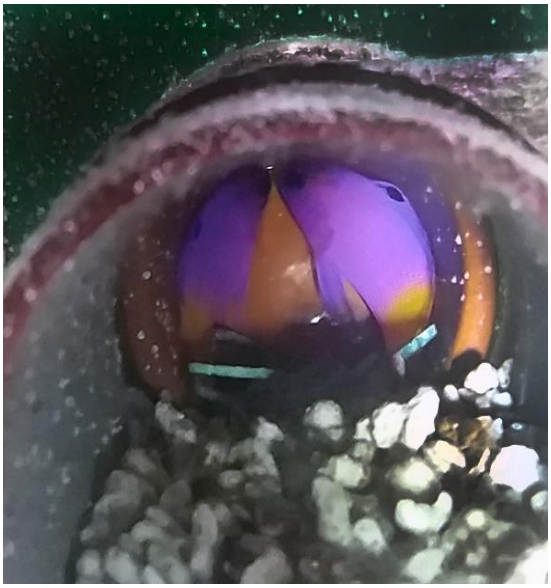
In nature, the species reproduces from February to June and a second period in October (Asoh, 1996)[7], with spawning occurring at dawn. In my facilities, G. loreto has been breeding throughout the year. Breeding pairs have alternated long periods in which they spawn assiduously with shorter periods of "rest". Spawning has occurred most frequently in the early morning (between 6 and 9 am), but also in the middle of the day. For more than three years, the species has spawned indistinctly, both in community aquarium and in specific tanks. In a 700 L community aquarium with plenty of natural refugia, one male and four females have produced a higher number of eggs than any of my other isolated pairs, even though the group of females quickly becomes hierarchically structured and not all of them have the same opportunities to spawn with the male. Even so, in a maintenance oriented to their reproduction, my preferred option has been to place adult pairs in specific aquariums of 150 liters. These aquariums are high enough to have a rocky wall full of shelters.

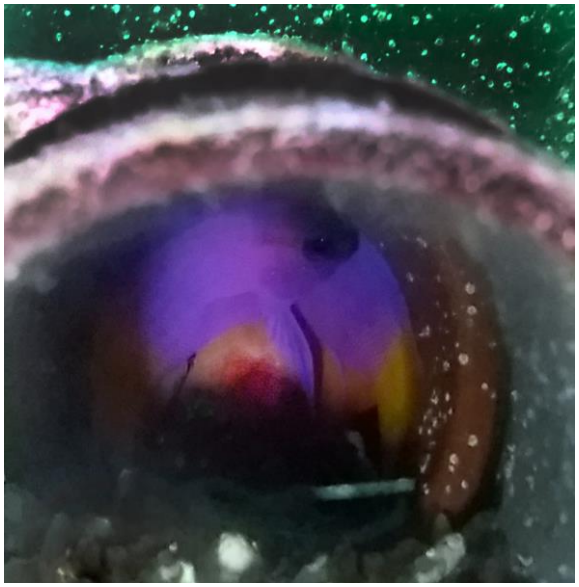
G. loreto. Males build nests in all types of shelters. Note the mass of eggs inside.

The male of *G. loreto* carries in its mouth algae and pebbles with which to build its nest.

Each male of *G. loreto* has its own "architectural style".

The shelters I use are designed to facilitate egg collection. They are revisable and interchangeable. They are composed of three pieces: a PVC plug with a diameter of 5-6 cm, a cylindrical tube of the same material and diameter and 9-10 cm in length, which is slightly embedded in the plug and finally a disk of green fibrous synthetic material attached to the bottom of the plug, and that will be the preferred surface for couples to make the spawning on it, remaining the eggs fixed to this easily interchangeable substrate, without the need to remove the shelter. In fact, egg attachment has peculiar characteristics. The egg, with a diameter of 1 mm and negative buoyancy, presents in the area surrounding the micropyle a series of chorionic "appendages" and also non-adhesive elastic filaments. These filaments are "entangled" in the chorionic appendages and in the disc of fibrous material, so that the egg cluster is well entangled with each other and with the spawning surface and with enough space for a good circulation of water to oxygenate them (Asoh- Yoshikawa, 1996)[8].

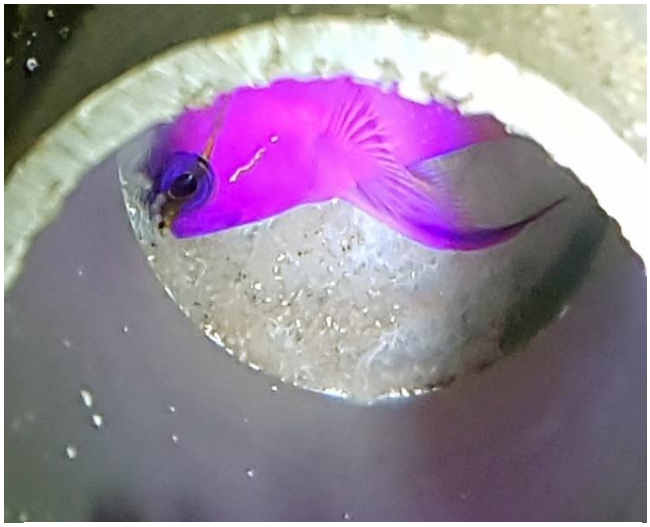




Two snapshots of a pair of *G. loreto* spawning inside the male's nest.

As mentioned in the introduction, G. loreto is a species with separate sexes from the juvenile stage. Although the species is described as having "no secondary sexual characteristics" that discriminate between males and females, the truth is that when one becomes familiar with the species, it is easy to distinguish between adult specimens, the males, which are generally more slender and with somewhat longer pelvic fins, while the females tend to be specimens of somewhat more rounded outline, less developed pelvic fins and somewhat more confident behavior. In very young specimens, where these characteristics are less evident, we can rely, when forming pairs, on their intense intraspecific aggressiveness. Thus, if you acquire a group of juveniles and allow them to interact in pairs, every time you put two males together they will start a confrontation, grabbing each other firmly by the jaws, and it will be necessary to separate them quickly to avoid serious damage, since when the aggressiveness increases, violent head shaking can damage them or the stronger specimen can bite on the body and tear the fins of his opponent.

A male of *G. loreto* interposes his body to protect the spawn, which he will care for throughout embryonic development.



In the picture, a male cleans and relocates the eggs to the bottom of the nest.

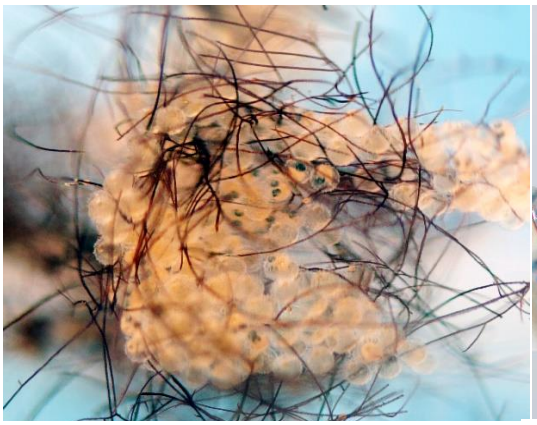
Once the breeding pairs are formed, they receive three to four feedings per day, following the criterion of "small quantity, frequently and varied". At least one of the intakes consists of live food (Artemia or mysidaceans), the rest of the intakes consist of some of the foods already described in the previous section. Adult males have reached an average of 7.5 cm LT and females about one centimeter less. The quality of the water is always high. The values normally measured in the breeding aquarium system have been: PO_4^-: 0,1 mg/l, NO_3^-: 10 mg/l, KH: 7ºd, S:33 g/l, pH:8,1, T:25-26,5ºC. The aquariums are provided with a light (10,000 K) that is not particularly bright for 14 hours and an ambient "moonlight" in the aquarium room at night.

When females are gravid, they come to the vicinity of the male's shelter and start the courtship, with characteristic movements that end up capturing the male's attention, which allows them to enter the shelter to spawn, proceeding immediately to the fertilization of the eggs. On other occasions it is the male who courts the female, going to look for her, "paralleling" their bodies with

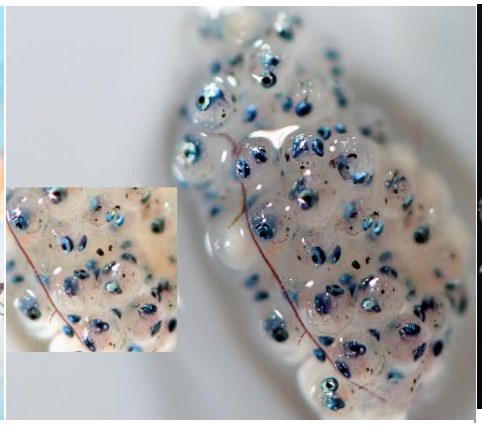
their fins extended, and then swim together to the vicinity of the shelter containing the nest. In my aquariums, the males have shown a great variability in the elaboration of their "nests" inside the shelters. There have been males with a great "artistic spirit" that with pieces of macroalgae and pieces of the substrate have built large nests that have not stopped to refurbish and repair, while others have been content to transport to the entrance of the shelter some small pebbles of coral sand.

Whenever a male has a clutch inside his shelter, he devotes all kinds of care to it: protection, cleaning, ventilation, nest reconstruction, etc. I have observed that some males feed irregularly during this period, not always leaving the shelter when food is offered.

An added difficulty in the development of larvae of this species lies in the way G. loreto spawns. During the spawning periods described above, the female deposits a small number of eggs each day, between 20 and 50 eggs, depending on the size and condition of the female. After 5-6 days, when spawning is complete, the refugium may contain an egg mass of between 100 and 275 eggs, with some sections in the early morula-blastula stage while other portions of the egg mass have half-formed embryos. This form of spawning has implications. The most important is the hatching of the larvae in small groups for about a week, instead of having all the offspring at once. This means that within the school there are notable differences in age and therefore in feeding requirements. It also makes it difficult to evaluate how well or badly the process is going, since, due to new hatchings, there is a daily "renewal of larvae" from the hatchery in the larvarium. At a temperature of 25ºC, I have a record for a single incubated egg mass of 10 consecutive days with daily hatches.



Males of *G. loreto* intertwine the egg mass with the nest algae.



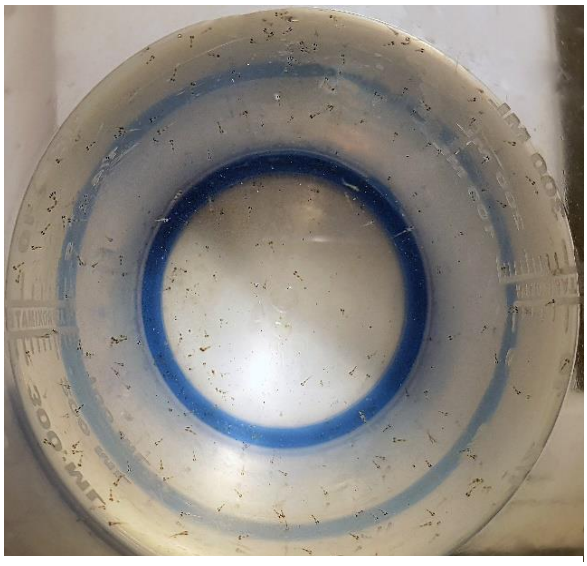
After 8-10 days, a new generation of *G. loreto* is ready to hatch.

The eggs of *G. loreto* are attached to each other by flexible, non-adhesive filaments.

The nests are inspected visually every day, to evaluate the progress of the spawning, but the fibrous disc that supports the egg mass is not removed, until most of the eggs present an embryo in advanced stage of development, in this sense and as it is well known, the state of formation of the eyes is the optimal indicator. If they are removed prematurely, the portion of eggs that will be spoiled will be significant. The fibrous disk with the egg mass is always collected submerged and transferred to a two-chamber incubator, inside which, well oxygenated, they will continue their development until hatching.

The larvae, at a temperature of 25-26.5°C, hatch after 8-10 days of embryonic development. Hatching always takes place at night. In my installations they start hatching 2-3 hours after the aquarium lights are turned off.

Once the larvae are detected, they are transferred from the hatchery to the larvarium. I capture and transfer the larvae with the help of a special pipette that does not damage them. The newly hatched larvae have fully formed eyes, an open mouth with well-developed jaws, a spiral digestive tract, light pigmentation and a minimal yolk sac that will keep them alive for only a few hours if they do not find suitable prey soon and in large numbers. My pairs have produced larvae ranging in size from 2.8 to 3.2 mm TL. Measurements of 2.9 to 3.8 mm TL are cited in the literature. (Asoh y Yoshikawa 1996)[8].



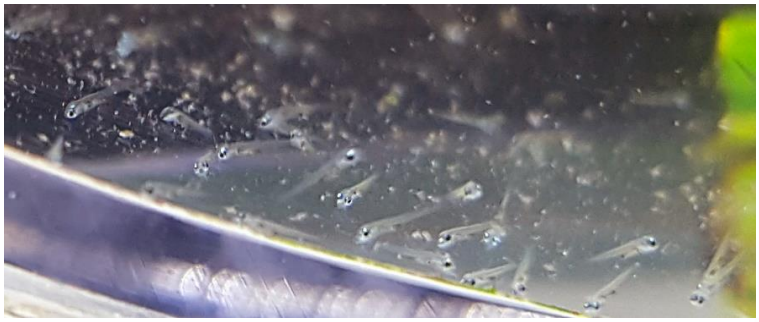
Larvae of *G. loreto* collected after hatching in the incubator.

Gramma loreto larva 6-8 hours old

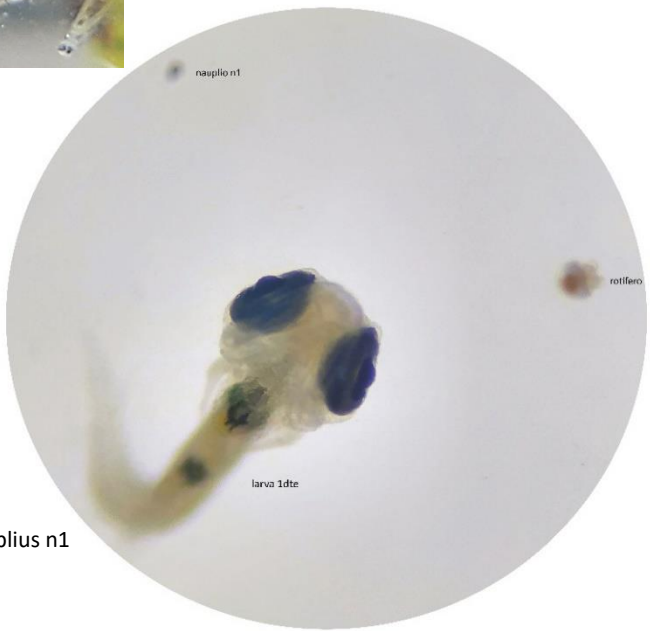
Development of larvae and fingerlings

The development system used is simple and responds to a well-known "classic" system. It is handmade and I have been modifying it as I have detected inefficiencies. It consists of four interconnected and interchangeable sections, according to the needs of the developing species. There is a "larval development section" to which I can incorporate a "Kreisel", cylindrical dark colored basins or a rectangular larval "tower", all of them of reduced volume (6-12 L). This section receives water from the "filtration section" (mechanical, chemical and biological) and then the water returns to the filter. There is a "fry section" where the most advanced specimens that have begun to settle and are in full metamorphosis are incorporated. It is based on cylindrical containers of different sizes (50, 100, 150 l), all of which are interchangeable. This section receives water from the same filter as the larval section, so that the transfer of specimens from one section to another does not involve a "change of medium". The system is completed with a UV "sterilization section", which returns the water through the return circuit to the larval and fry sections. The larval section evacuates water into the system through planktonic meshes with porosity between 100 and 200 microns (depending on species and initial diet). Below 100 microns the system is not very

operational due to frequent clogging of the mesh. However, I must admit that the use of planktonic evacuation meshes with pores of 100 - 200 µ diameter has some drawbacks, as we will see below.



Larvae 1dph feeding in development system



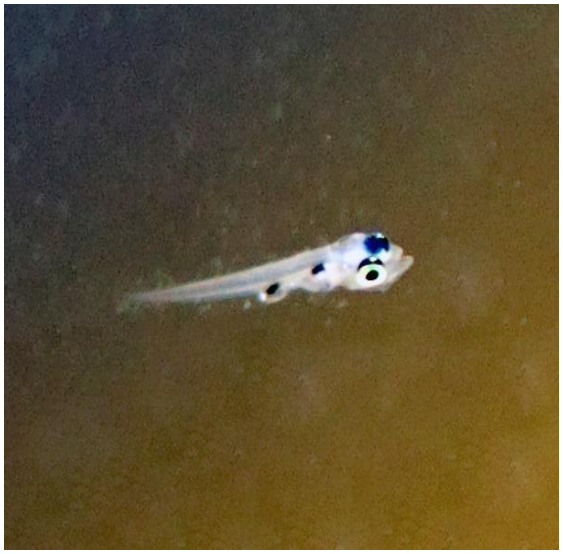
Size comparison. Larva 1dph vs. nauplius n1 of *Apocyclops* vs. *B. rotundiformis*

Once the larvae are already in the development system, they are fed as soon as possible. The initial diet was based on the rotifer *Brachionus rotundiformis* and N1 nauplii of the copepods *Acartia tonsa* and *Apocyclops panamensis*. In my cultures, *B. rotundiformis* reached an average size of 180x110 µ (LxW) and the N1 nauplii of the mentioned copepods reached a size of 100x70 µ (LxW). Additionally, a variable volume of the microalgae *Tisochrysis lutea* and *Tetraselmis suecica* was added to the larvarium. In this initial diet, with some groups of larvae, it has been tried to substitute *T. suecica* by a diatom (*Phaeodactylum tricornutum*), but the results have been worse. I have also occasionally used N1 nauplii of the copepod *Parvocalanus crassirostris* (65x50 µ. LxW), but the small size of my cultures of this species and the not very high fertility rate have unfortunately made their use anecdotal.

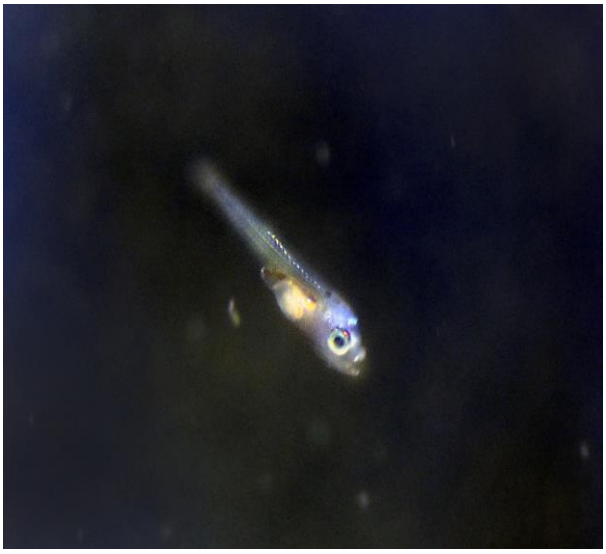
Each time food is supplied, it is previously "enriched" in a 50% mixture of the two microalgae already mentioned; the rotifer for 8-12 hours and the N1 nauplii for 15-20 minutes. The general idea has been to maintain an average concentration of 6-12 rotifers ml^{-1} and 0.5-1.5 nauplii ml^{-1}. Two samplings per day are carried out to determine the existing food concentration.

The initial larvarium conditions were: medium-intense light 24 hours, soft aeration line directed towards the best illuminated area, S:30-32 ppt, T:26ºC. In closed circuit without "dripping" from the filter to be able to feed the larvae with almost no leakage of food and microalgae.

During the first days, it is observed that the larvae are quite static, they position themselves in mid-water near the walls of the larvarium and their movements are very short. They do not move from end to end of the larvae looking for food, but wait for the food to pass close to them, for which they preferably position themselves in the best lit area and against the current; I think they can be considered at this early stage as "semi-passive predators" and this conditions the concentration of food in the larvae. In any case, the larvae seem to feed from the first day.



G. loreto. Larvae 5 dph.



G. loreto. Larvae 14 dph.

Of the two mortality peaks usually observed during the development of these larvae, the first is very high and occurs during the first three or four days. I think that the three main causes are: bacterial infections (*G. loreto* larvae seem to be very sensitive to pathogenic bacteria), poor genetic quality of the larvae, and finally insufficient or non-homogeneously distributed food in the larvarium.

Towards the end of the first week, a partial water renewal by drip of 1 l/h is started for a few hours per day. From that moment on, nitrites, which are a recurrent threat, stabilize around NO_2^- = 0.07 ppm. At the age of 7 dph, a slight growth is observed in the surviving larvae, reaching 4mm TL.

During the second week the same diet and food concentration is maintained regardless of the number of surviving larvae. Larvae at 14 dph show a black line on the flank and under binocular magnification slight color iridescence, their average size is 6 mm TL.

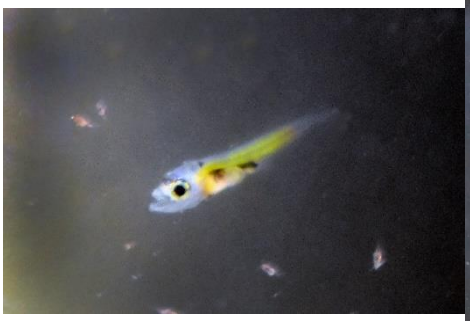
The second significant peak in mortality occurs between the end of the second week and the middle of the third week, probably coinciding with the delicate "flexion" phase.

During the third week, C1 copepodites (300-350 µ) of the two copepod species mentioned above and occasionally also *P. crassirostris* are incorporated into the diet. At 21dph, the larvae show

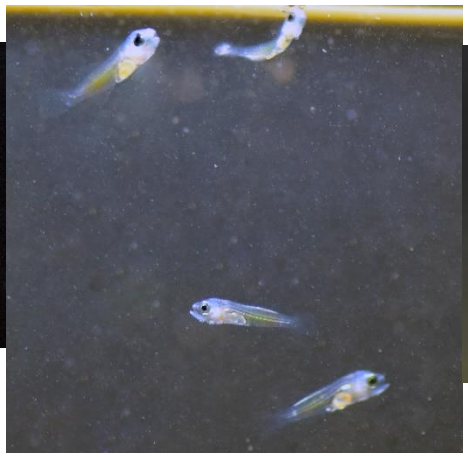
lemon-yellow and orange-reddish pigmentation in macrophotographs. Their size reaches on average 8 mm TL.

Constant water renewal by drip from the filtration-sterilization section is introduced from day 22 dph, paying special attention to "feed leakage" from that moment on.

It is during the fourth week that remarkable vertical growth occurs. By this time the larvae are acquiring the ability to capture larger copepodites C2-C3. At 28 dph they already show some benthic habits, although the definitive "settlement" has not yet taken place. Their average size reaches 1 cm TL.



G. loreto. Larvae at 16 dph.



G. loreto. Group of larvae at 18 dph.

G. loreto. Larvae at 21 dph.

As the larvae evolve, size differences between them become apparent, due in part to the daily cadence of hatching already described; but no aggression or cannibalism by the larger larvae towards the rest has been observed.

Throughout the fifth week of development, newly hatched nauplii of *Artemia franciscana* are gradually introduced into the diet. I know that other breeders of this species introduce this food earlier, but in my case, it has not been positive to anticipate it and I have preferred to continue with nauplii and copepodites of the species already described. In this phase, the 24-hour lighting is replaced by 16 hours of daylight and 8 hours of "moonlight" emphasizing the bluish spectrum. It is during this week when the "settlement" has usually taken place, being possible to observe specimens in inverted position, linked to improvised shelters. The black ocellus at the beginning of the dorsal fin is visible to the naked eye and at the age of 35 dte they already measure an average of 1.3 cm TL.

G. loreto. Fry 30 dph

G. loreto. Fry 35 dph

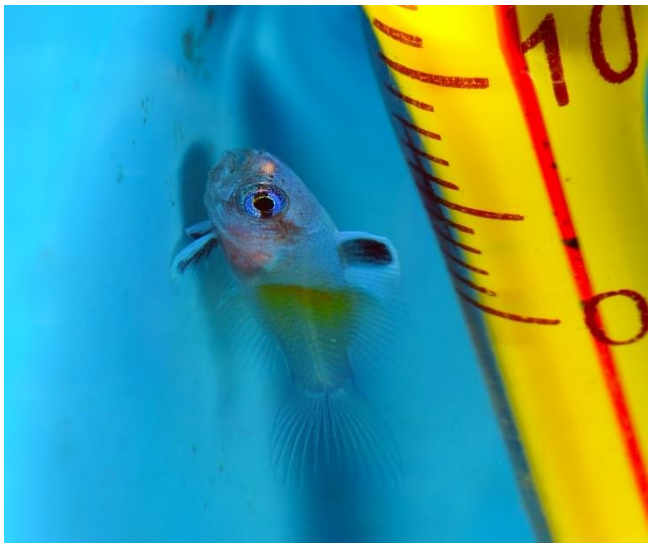
Over the next two weeks, they progressively broaden their feeding spectrum, first incorporating *Artemia* meta-nauplii (previously enriched for 12 hours) and then adult copepods of *Apocyclops panamensis*. In order not to make this description too long, I leave in Table 1 a detailed summary of the whole feeding protocol.

In this period, and already transferred to the fry section, the growth rate accelerates, measuring an average of 1.6 cm TL at 47 dph. Metamorphosis is at a very advanced stage. First the lemon-yellow coloration of the posterior part of the body is accentuated and a few days later the purple coloration of the anterior part. In this phase, the pelvic fins, pigmented light blue and black, divided into two longitudinal stripes, are striking in their appearance. Also, the black ocellus of the dorsal fin is bordered with a delicate light blue circle.

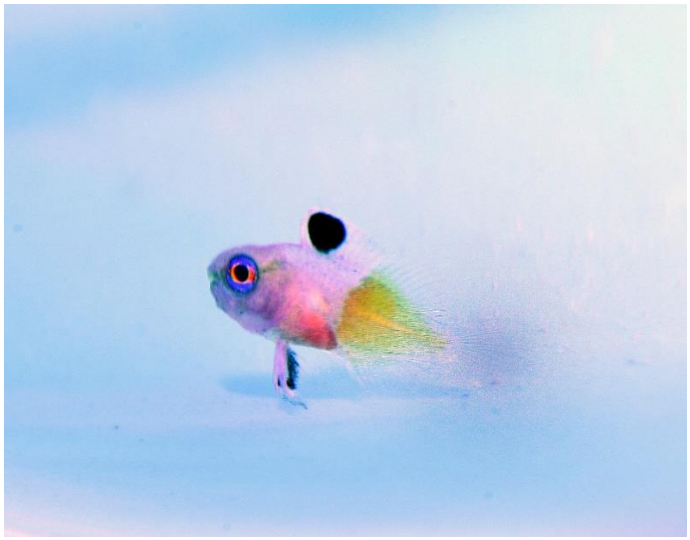
With the described protocol, metamorphosis has been reached by the earliest specimens in 50 dph and the later ones in 60 dph. By this time, the fry have been actively exploring the surroundings of their shelters for some time. At two months of age they are approaching 2 cm and at three months 2.5 cm and are already distributed in aquaria for juveniles or for the formation of breeding pairs.

Gramma loreto. Larval feeding protocol	W1	W2	W3	W4	W5	W6	W7
Acartia tonsa-nauplii N1 (100x70 µ) Apocychlops panamensis -nauplii N1 (100x70 µ) Parvocalanus crassirostris- nauplii N1 (65x50 µ)	◊◊◊	◊◊◊	◊◊	◊			
Brachionus rotundiformis (190 x 120µ). Enriched.	◊◊◊	◊◊◊	◊◊				
Brachionus plicatilis (320x 170 µ). Enriched.			◊◊	◊◊	◊◊		
Acartia tonsa copepodite C1 (350 µ) Aphocychlops panamensis copepodite C1 (300 µ) Parvocalanus crassirostris copepodite C1 (250 µ)			◊◊	◊◊◊	◊◊◊		
Artemia franciscana-nauplii<6h (500 µ)					◊	◊◊◊	
Artemia salina-metanauplii (650 µ) (Enriched with EDS® + Tisochrysis lutea) Acartia tonsa adult 1200 µ Aphocychlops panamensis adult 700-800 µ Parvocalanus crassirostris adult 400-500 µ							◊◊◊
Age	1-7dph	8-14dph	15-21dph	22-28dph	29-35dph	36-42dph	43-49dph

The diamonds represent the importance of that food during the time indicated in the column. Three diamonds represent indispensable food in the diet. One diamond represents food that is either beginning to be consumed or is beginning to stop being consumed. In both cases it is not the main component of the diet for that time



During settlement they improvise all kinds of shelters. Fry at 45 dph.



G. loreto. Fry at 55 dph

G. loreto. Young specimen of 60 dph

Juveniles and sexual maturity

Groups of juvenile *G. loreto* can develop normally in aquariums of about 150 liters well provided with pvc shelters and / or in natural rocks, and with abundant planting of macroalgae, such as *Caulerpa prolifera*. The dense vegetation together with the profusion of hiding places makes direct vision difficult and reduces skirmishes, although it is true that, although still moderate, an intraspecific aggressiveness to watch is already visible.

From a size of 2 cm, they are already fed with a conveniently chopped adult diet and some flake food. At about 3 cm they can begin to be distributed in pairs or reproductive groups, although it will still take a few months before they produce their first small, fertile spawns. In nature, the smallest sexually mature males and females measured were 3.28 and 2.53 cm in total length, respectively. (Asoh y Shapiro 1997)[3].

G. loreto. Young specimens 4 months old

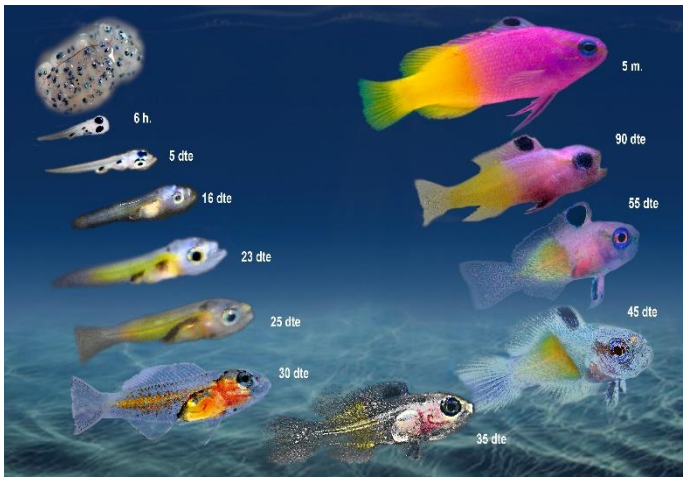
Some final considerations

I believe that I have not left anything relevant to describe in relation to what I have learned while reproducing this species for some years in my small facility dedicated to reproduction. I must admit that my experience "clashes" partially with that part of the reproductive literature of the species that states that larvae are "easily" developable only with rotifers. This has not been my experience. Every time I have approached the development of a group of these larvae with an initial diet based exclusively on rotifers, I have not been able to get the larvae to complete their metamorphosis. It has always been necessary to add to that initial diet, N1 nauplii of one of the three species of copepods already described. Some research centers have had the same experience as I have had regarding the composition of the initial diet. (Ospina-Salazar,2011)[10].

Another aspect to consider, because of their potential impact on "mortality peaks", are the bacterial infections that these larvae often suffer and to which they are especially vulnerable. In general, pathogenic bacteria can come from the larvarium's own water and/or from food based on live prey. As for the larvarium water, as already described, in my case well filtered and reasonably sterilized water is available, but during the first days of development, the larvarium section does not receive permanent water renewal by drip. Therefore, bacterial proliferation is more than likely and only the bactericidal capacities of the microalgae added, especially *Tetraselmis*, will be able to limit their exponential growth. As for food, the quality and healthiness of the cultures is fundamental in the survival rates of *G. loreto* larvae, especially with regard to rotifer cultures and later *Artemia* nauplii. To emphasize this aspect, a single rotifer can carry up to 1,000 bacteria and an Artemia nauplius can carry up to 10,000 bacteria. (G. Giménez-F. Padrós, 2006)[11].

Finally, the state of "maturation" of the larva at the moment of hatching seems to me to be a relevant aspect. I have tried to work on this concept, incubating some clutches at a lower temperature (24ºC) than usual, thus slightly lengthening the time of embryonic development. I have also incubated another set of clutches at a higher temperature (27.5ºC) than usual (25ºC), obtaining embryonic development times slightly shorter than the average value. The average survival was apparently somewhat higher in the larvae of the first case, with longer embryonic development times. And I say apparently, because the number of variables to be controlled in any larvarium

development process is sufficiently high, so that the results cannot be categorically attributed to only one cause.



G. loreto. Graphical summary of its development.

In the same way that mariculture feeds us in an efficient way, aquaculture of ornamental species helps us to preserve aquatic biodiversity. In this context, and despite the fact that *G. loreto* is assessed by the IUCN as "LC" (Least Concern), I sincerely think that it is not a bad idea to continue optimizing its reproduction in controlled environments.

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