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IRCP Report – Grant Tahiti Perles

**Study of visual abilities of coral reef fish larvae
in relationship with conspecifics, predators, light intensity of light traps
and their brain organization**

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Ambassade de France à Fidji

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1) General introduction

The biodiversity is declining, and habitat destruction and degradation are now commonplace. Examples of degradation can be found throughout marine ecosystems, including estuaries, salt marshes, soft-bottoms, hard-bottoms, and coral reefs (Hughes *et al.* 2003). The degradation in coral ecosystems is usually characterized by coral mortality from natural and anthropogenic stressors (e.g., disease, hurricane damage, pollution, temperature-induced bleaching). This decrease of coral cover opens space on most reefs and causes substantial increases in cover and biomass of rapidly growing fleshy and filamentous macroalgae which, in turn, limits the recovery of coral populations and then modify fish and invertebrates communities (Edmunds & Carpenter 2001). Thus, areas experiencing perturbation often exhibit declines in adult populations, leading to a higher rate of extirpation than in pristine habitat, and the persistence of species in the area becomes reliant on the "rescue" effect of recruitment (Hanski & Gilpin 1997). The potential of the area's population to be supplemented by recruits, however, depends on whether pelagic larvae detect an appropriate habitat in that area and then settle and persist in that habitat (Sale 2002).

The coral reefs in the Pacific have been ravaged and demand is ever increasing to match the global and domestic demand for the food fish and crustacean market and for the lucrative aquarium trade (Hair 2000). Without sustainably managing these resources, loss of diversity and extinction are certain (Nystrom *et al.* 2000). People are now turning to catching and growing aquatic organisms and plants to increase food security and economic benefit (Bell *et al.* 2009). Studies spanning more than a decade in French Polynesia have shown that along with effective capture techniques, there is also a need for efficient and practical capture and culture practices to make the aquarium trade and reef restocking schemes a success (Bell *et al.* 2009).

Thus, the abundance and diversity of coral reef fish depend mainly on the recruitment success (Sale 2002), which is characterized by important seasonal and inter-annual variations (Doherty & Williams 1998). Recruitment success depends, for a large part, on the number of survivors among pelagic larvae in the water column (Boehlert 1996). The life cycle of coral reef fishes includes two major phases - a planktonic phase and a demersal or benthic phase. Coral reef fish spawn to produce eggs and larvae that disperse into the open ocean. After development as plankton, larvae recruit back to reefs. Most coral reef fishes have complex life cycles, involving the broadcast of eggs or larvae into the water column (Fontes *et al.* 2009), fertilized eggs disperse into the open ocean. Other species lay demersal eggs into nests, and then guard them until they hatch. Some species guard their young after

hatching as well, but many simply allow the newly-hatched yolk sac larvae to disperse into the water column - where they enter the other major life cycle phase (planktonic larval phase). The planktonic phase is spent in the open ocean; often well away from any reefs. The planktonic phase usually lasts a few weeks to months. After the larval fish runs out of yolk, it eats plankton smaller than itself, such as diatoms, dinoflagellates, and copepods. Larval fish in turn are eaten by larger plankton, such as jellyfish, chaetognaths, and larval crabs. Assuming that the fish does not starve, get eaten, or become lost on the wrong ocean currents, near the end of its larval duration it begins searching for a suitable reef upon which to settle (or recruit).

Among the various methods that can be utilized to study marine larval supply, (e.g., light traps, crest nets, artificial habitats and dip or semi-submersible nets (Carassou 2008) crest nets and light-traps have one major advantage over other methods: marine larvae are caught immediately prior to settlement, which would give a suitable measure of larval supply (Lecchini *et al.* 2006). Thus, the most commonly used sampling method for PCC is the use of light traps and crest nets as they are capable of retaining the specimens alive and in good condition (Bell *et al.* 2009). In my Master project, light-traps were used that captured fish larvae which are phototropically positive (Leis & McCormick 2002). The overall objective of the present study is to improve the knowledge on visual abilities of coral reef fish larvae in order to improve, in the future, the efficiency of light traps.

2) Efficiency of light intensity of light traps to capture coral reef fish larvae

2a) Introduction

There have been many techniques experimented with that attempt to maximize larval fish and crustacean yields, such methods which include using chemicals, light traps, crest nets, mid-water net tows, underwater seining and the use of artificial reef habitats (see in Dufour & Galzin 1993, Lecchini *et al.* 2004, Carassou *et al.* 2008). Each aforementioned larval sampling technique aims to target the various stages of larval development. For example, crest nets are aimed at catching settling larval fish and crustacean before colonization whilst light traps are aimed at obtaining pre-settling fish and crustacean larvae and artificial reef habitats target juveniles that have found a suitable substrate and have colonized (Carassou *et al.* 2008). Several studies have shown that the majority of oceanic ichthyoplankton are phototropic and this attraction to light is common to many marine organisms, but disappears when the young fish are settled (Leis & McCormick 2002). Not all marine fish larvae can be caught in light traps as this method of capture is only effective if the target species is positively phototropic (attracted to light). Sieback & Marshall (2007) showed that fish larvae that were caught in light traps used well-developed ocular media in the detection of their prey. Larvae use UV vision as an aid in planktivorous feeding, contrast enhancement against the ocean as a background, navigation and orientation (Lara 2001). Nevertheless, there is still a lack of research on the effects of light intensity on fish behaviour in order to improve, in the future, the efficiency of capture of light traps. This is the objective of my first study of Master.

2b) Methodology

Fish larvae collected with light-traps at Moorea were used to better understand the light attraction of species. A larva was placed in the center of aquarium with 4 compartments. Each compartment had a different level of light intensity (Fig. 2.1). A light with 52 LED linked to 12V battery (same level intensity that in light trap) was set up in one compartment. This system allows to have different level intensities in each of the four compartment. The larva introduced in the center of aquarium could move in the four compartments and when a larva spent more than 30 seconds in one compartment, the experiment was stopped. After 3''30', if no larva spend more than 30' in one compartment, the experiment was stopped and noted « no choice ». Two trials were conducted on each

larva: 1) control trial with no light in aquaria, and 2) test trial with light. The objective is to compare by a Chi-square test the theoretical distribution (control trial) to the observed distribution (test trial).

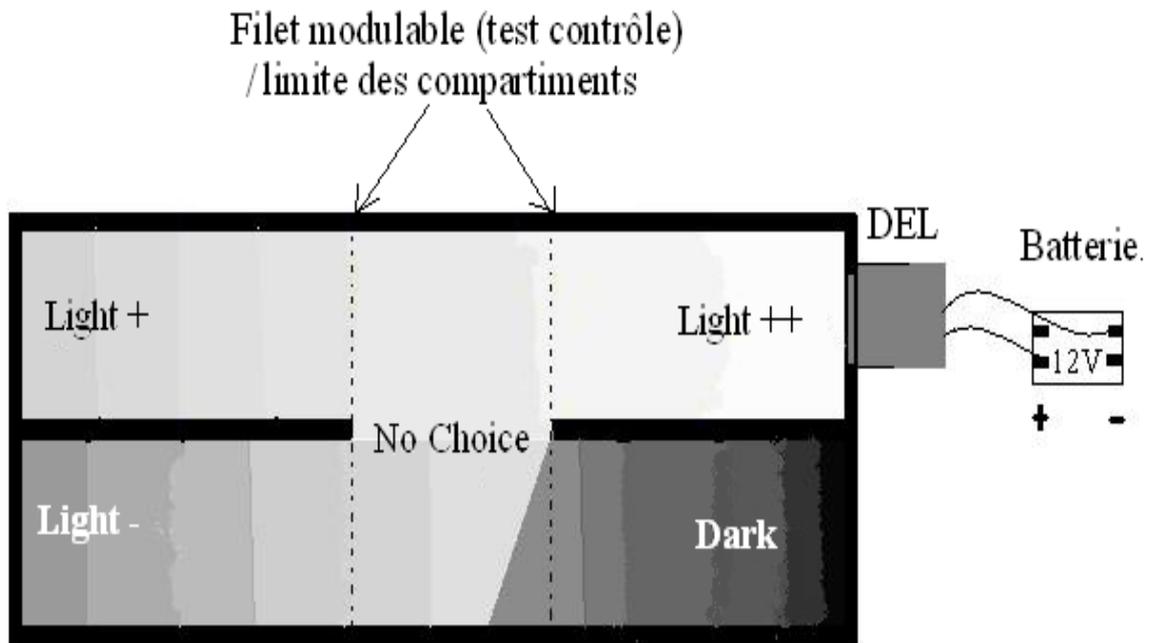


Figure 2.1: Aquarium design to test the larval attraction to different levels of light intensity (*Light ++*, *Light +*, *Light -*, *Dark*).

2c) Results and discussion

A total of 7 species was tested with 5 to 59 larvae by species. Friedman test conducted on both 7 species showed that fish larvae had a different distribution in absence or presence of light ($\chi^2 = 738$; $p < 0.001$). Thus, 3% of larvae were attracted by the brightest compartment (L++) and 40% by the second less bright compartment (L-) (Figure 2.2). At species level, χ^2 test showed that 3 of the 7 species were significantly attracted by one of the four compartments (Tableau 2.1).

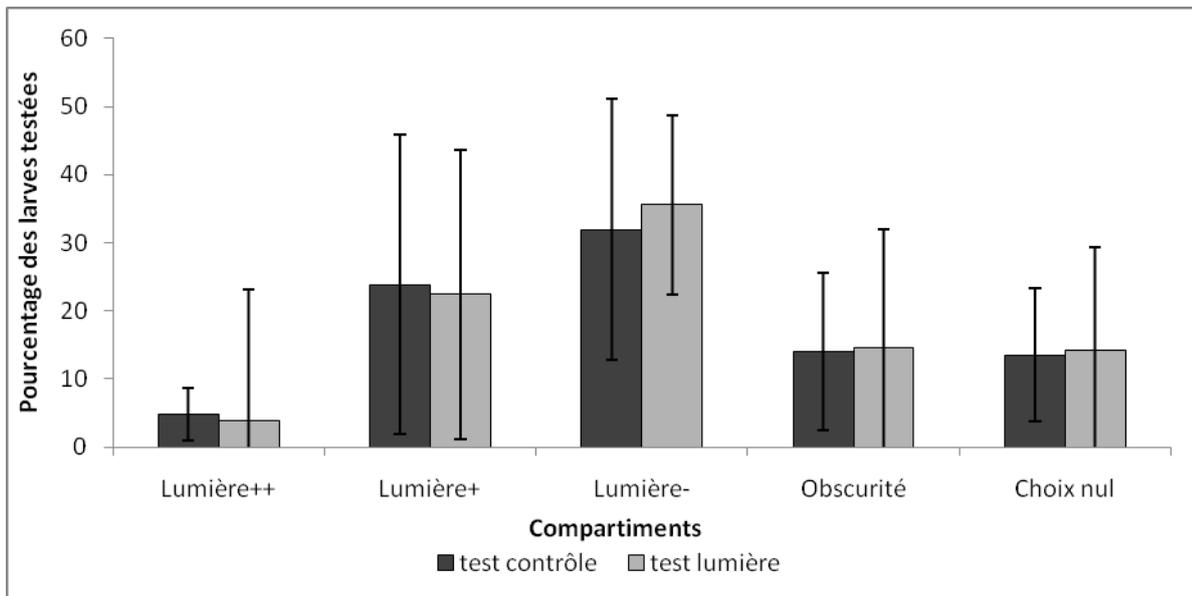


Figure 2.2: Distribution (in %) of coral reef fish larvae among the four compartments of aquarium in control trial (without light) and test trial (with light). NC means fish larvae stayed in the central part of aquarium.

Table 2.1: Results of χ^2 test conducted on the 7 species to highlight if one of the four compartments attracted significantly the fish larvae.

Species	χ^2 -value	P-value	Most attracted compartment
<i>Myripristis Kuntze</i>	58,2	p<0,001	L- (significant)
<i>Myripristis Pralinia</i>	1,36	0,85	L- (not significant)
<i>Neoniphon argenteus</i>	2,50	0,62	L- (not significant)
<i>Neoniphon sammara</i>	2,00	0,69	L+ (not significant)
<i>Ostorhinchus augustatus</i>	7,33	0,16	L+ (not significant)
<i>Sargocentron microstoma</i>	21,0	p<0,001	L- (significant)
<i>Sargocentron spiniferum</i>	16,5	p<0,001	L- (significant)

3) Visual abilities of coral reef fish larvae in the recognition of conspecifics and predators

3a) Introduction

After reaching a reef, fish larvae have to choose a settlement habitat based on the presence or absence of specific benthic substrates, conspecifics, heterospecifics and predators (e.g. Booth & Wellington 1998, Lecchini *et al.* 2005a, b, 2007). Even though long distance visual navigation is impossible due to limited visibility in water, fish larvae could use visual cues for microhabitat selection. Larval fish have excellent vision (Job & Bellwood 2000, Job & Shand 2001), and species that settle during that day have been found to use their eyesight to avoid habitats with potential predators (Leis & Carson-Ewart 1999) and select habitat with conspecifics (Booth 1992). Thus, some physiological studies have demonstrated that fish larvae had good visual abilities, but these abilities could improve after metamorphosis for some species (Kotrschal *et al.* 1990, Lara 2001). In this study, experiments were conducted on coral reef fish larvae to determine: 1) which species are attracted to conspecifics using visual cues; 2) which species are repelled by predators using visual cues.

3b) Methodology

To explore the visual cues, experiments were conducted at night (but with lights in experimental room) with fish larvae captured with crest nets. Ten different larvae for each species were used for Experiments 1a and 1b. Each experiment used a “choice chamber” whereby only visual cue was available. Two experiments were conducted and each forced on different options (Experiment 1a: conspecifics vs. heterospecifics; Experiment 2b: predator vs. nothing).

To assess visual cue, the factor (e.g. conspecifics or predators) were placed in tanks adjacent to the choice chamber, but placed on separate Styrofoam blocks to isolate auditory or vibratory cues (e.g. in Tank 1 vs Tank 2, Fig 3.1). The choice chamber was partitioned using white solid blocks which were approximately 10 cm wide and 11 cm high. For each trial, one fish would be released into the central compartment (A, Fig 3.1) for a acclimatization period of 1 minute after which the partitions would be raised simultaneously and the time the fish spends in each compartment (A, B and C, Fig 3.1) was recorded (Experiment time of 1 minute was allowed). Compartment A was always towards the heterospecifics or predators, while compartment C was always towards the conspecifics.

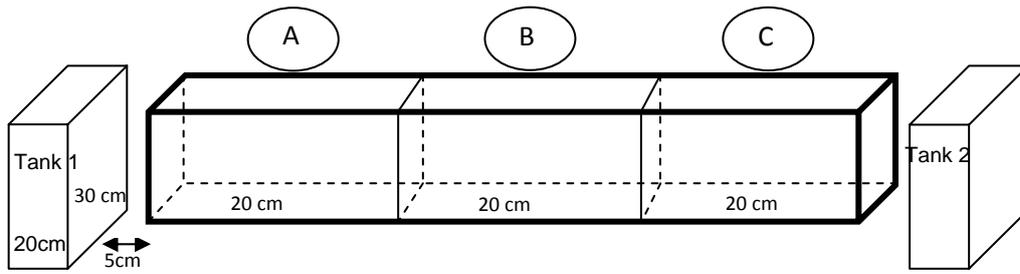


Figure 3.1: Diagram of choice chamber used to evaluate visual cue. The chamber consists of an aquarium with three compartments, with A, B and C partitioned with white solid blocks of width 10cm and height of 11cm. Additional aquaria on either side of the choice chamber (labeled tank 1 and 2) are isolated and mounted on separate Styrofoam to prevent transfer of vibratory signals.

- Experiment 1a: Attraction to Conspecifics

The objective of Experiment 1a was to determine which coral reef fish larvae are attracted to conspecifics when only visual cues were available. Ten fish of each species were used for this experiment, involving three treatments (control, heterospecifics vs. nothing, and heterospecifics vs. conspecifics). A maximum of six fish of each species were used as conspecifics and a maximum of six *Chromis viridis* were used as heterospecifics. Water was replaced for each trail (to isolate chemical cues) and the positions of treatments were randomized for each trial.

- Experiment 1b: Effect of Predators

The objective of Experiment 1b was to determine which species recognize and are repelled by predators when only visual cues were available. Ten fish for each species were used (different fishes from that used in Experiment 1a) for this experiment, involving two treatments (control, and predator vs. nothing). One or two predators were used at a time. Some of the species used as predators included *Sargocentron spiniferum*, *Neoniphon sammara*, *Myrispristis kuntee*. As in Experiment 1a, the water was replaced for each trial and the positions of treatments were randomized for each trial.

To analyze the data, chi-square tests were conducted between the control distribution (trial 1 of each experiment) and the observed distribution (trial 2 or 3 of exp.1 and trial 2 of exp. 2). The statistical tests were not conducted at species level, but according to life traits of fish species. Indeed, the 24 species were grouped into three life traits: swimming abilities, body color at colonization and behavior (Table 3.1).

Table 3.1: List of species studied according to their life traits. For swimming abilities, “slow”: $< 20 \text{ cm.s}^{-1}$, “moderate”: $20 < v < 40 \text{ cm.s}^{-1}$, “fast”: $v > 40 \text{ cm.s}^{-1}$.

Famille (11)	Espèce (24)	n	Comportement Social	Couleur de la larve	Vitesse Nage
Scorpaenidae	<i>Scorpaenoides diabolus</i> (Cuvier, 1829)	20	Solitaire	Transparentes	Lente
	<i>Scorpaenoides guamensis</i> (Quoy & Gaimard, 1824)	20	Solitaire	Transparentes	Lente
Apogonidae	<i>Apogonichthys ocellatus</i> (Bennett, 1830)	30	Solitaire	Transparentes	Modérée
	<i>Ostorhinchus angustatus</i> (Smith & Radcliff, 1911)	30	Solitaire	Transparentes	Modérée
Mullidae	<i>Pristiapogon exostigma</i> (Jordan & Stick, 1906)	14	Solitaire	Transparentes	Modérée
	<i>Mulloidichthys flavolineatus</i> (Lacepède, 1801)	19	Grégaire	Juveniles	Rapide
	<i>Parapeneus barberinus</i> (Lacepède, 1801)	16	Solitaire	Juveniles	Rapide
Chaetodontidae	<i>Parapeneus multifasciatus</i> (Quoy & Gaimard, 1824)	5	Grégaire	Juveniles	Rapide
	<i>Chaetodon citrinellus</i> Cuvier, 1831	25	Couple	Juveniles	Rapide
Pomacentridae	<i>Chaetodon lunula</i> (Lacepède, 1802)	6	Couple	Juveniles	Rapide
	<i>Abudefduf sexfasciatus</i> (Lacepède, 1801)	16	Grégaire	Juveniles	Rapide
	<i>Chromis viridis</i> (Cuvier, 1830)	30	Grégaire	Juveniles	Modérée
	<i>Chrysiptera glauca</i> (Cuvier, 1830)	13	Grégaire	Juveniles	Modérée
	<i>Chrysiptera leucopoma</i> (Bennett, 1828)	30	Grégaire	Juveniles	Modérée
	<i>Dascyllus aruanus</i> (Linnaeus, 1758)	14	Grégaire	Juveniles	Modérée
	<i>Pomacentrus pavo</i> (Bloch, 1787)	20	Grégaire	Transparentes	Modérée
Labridae	<i>Stegastes fasciolatus</i> (Ogilby, 1889)	17	Solitaire	Juveniles	Modérée
	<i>Thalassoma hardwicke</i> (Bennett, 1830)		Grégaire	Transparentes	Lente
Gobidae	<i>Valenciaenna strigata</i> (Broussonet, 1782)	30	Couple	Transparentes	Rapide
Microdesmidae	<i>Ptereleotris microlepis</i> (Bleeker, 1856)	20	Grégaire	Transparentes	Modérée
Acanthuridae	<i>Acanthurus triostegus</i> (Linnaeus, 1758)	95	Grégaire	Transparentes	Rapide
	<i>Ctenochaetus striatus</i> (Quoy & Gaimard, 1825)	15	Grégaire	Transparentes	Rapide
Balistidae	<i>Rhinecanthus aculeatus</i> (Linnaeus, 1758)	20	Solitaire	Juveniles	Rapide
Tetraodontidae	<i>Canthigaster benneti</i> (Bleeker, 1854)	30	Couple	Juveniles	Modérée

3c) Results

- Experiment 1a: Attraction to Conspecifics

A total of 200 larvae belonging to 21 species were tested. Whatever the trials, fish larvae remained mainly in the central compartment of aquarium (Fig. 3.2). Nevertheless, when conspecifics were introduced in one of the two tanks, fish larvae moved more often toward this tank (Fig. 3.2). Thus, chi-square conducted behavior' life trait showed that fish larvae life alone were not significantly attracted by conspecifics ($p = 0.112$), while fish larvae life in school were significantly attracted ($p = 0.004$). The chi-square conducted color' life trait showed that fish larvae with transparent body were not significantly attracted by conspecifics ($p = 0.588$), while fish larvae with juvenile color were significantly attracted ($p = 0.079$). Lastly, chi-square conducted swimming abilities showed that fish larvae with low swimming abilities were not significantly attracted by conspecifics ($p = 0.658$), while fish larvae with medium or fast swimming abilities were attracted ($p = 0.012$ and 0.020 , respectively) (Table 3.2).

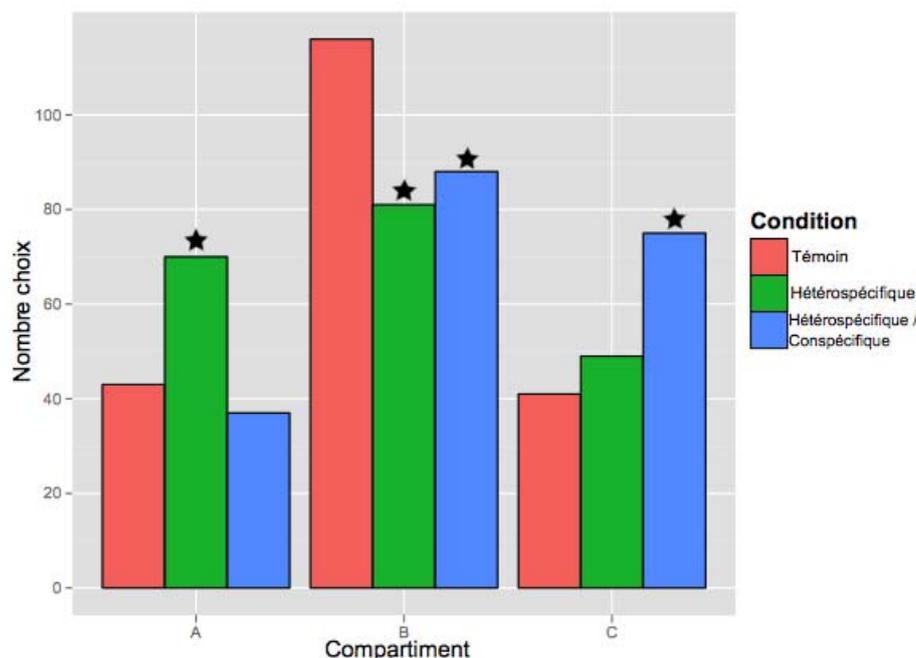


Figure 3.2: Distribution of fish larvae among the three compartments of aquarium according to the trials

Table 3.2: Results of Chi² tests according to the life traits of fish species at larval stage.

Critère	Catégories	Condition	n	A	B	C	A+B	B+C	X	p	ddl
Comportement social	Seul	Témoin	71	14	45	12	59	57			
		Hétérosppécifique	71	20	36	15	56	51	0.97	0.326	1
		Hétéro/Consppécifique	71	14	36	21	50	57	2.53	0.112	1
	Couple	Témoin	30	10	13	7	23	20			
		Hétérosppécifique	30	8	14	8	22	22	0.08	0.778	1
		Hétéro/Consppécifique	30	9	9	12	18	21	1.23	0.267	1
	Groupe	Témoin	99	19	58	22	77	80			
		Hétérosppécifique	99	42	31	26	73	57	11.47	0.001	1
		Hétéro/Consppécifique	99	14	43	42	57	85	8.33	0.004	1
Couleur de la larve	Transparente	Témoin	93	15	61	17	76	78			
		Hétérosppécifique	93	29	46	18	75	64	0.42	0.518	1
		Hétéro/Consppécifique	93	8	52	33	60	85	0.29	0.589	1
	Couleur juvéniles	Témoin	107	28	55	24	83	79			
		Hétérosppécifique	107	41	35	31	76	66	3.08	0.079	1
		Hétéro/Consppécifique	107	29	36	42	65	78	6.33	0.012	1
Vitesse de nage	Lente	Témoin	20	5	11	4	16	15			
		Hétérosppécifique	20	5	11	4	16	15	0.05	0.818	1
		Hétéro/Consppécifique	20	3	15	2	18	17	0.20	0.658	1
	Modérée	Témoin	105	16	75	14	91	89			
		Hétérosppécifique	105	27	53	25	80	78	2.92	0.087	1
		Hétéro/Consppécifique	105	13	57	35	70	92	10.65	0.001	1
	Rapide	Témoin	75	22	30	23	52	53			
		Hétérosppécifique	75	38	17	20	55	37	6.25	0.012	1
		Hétéro/Consppécifique	75	21	16	38	37	54	5.42	0.020	1

- Experiment 1b: Effect of Predators

A total of 201 larvae belonging to 22 species were tested. Whatever the trials, fish larvae remained mainly in the central compartment of aquarium (Fig. 3.3). Moreover, when predators were introduced in one of the two thanks, fish larvae did not move more often away to this thank (Fig. 3.3). Thus, chi-square conducted each life trait showed that fish larvae were not repulsed by the presence of predators (all p-value > 0.05).

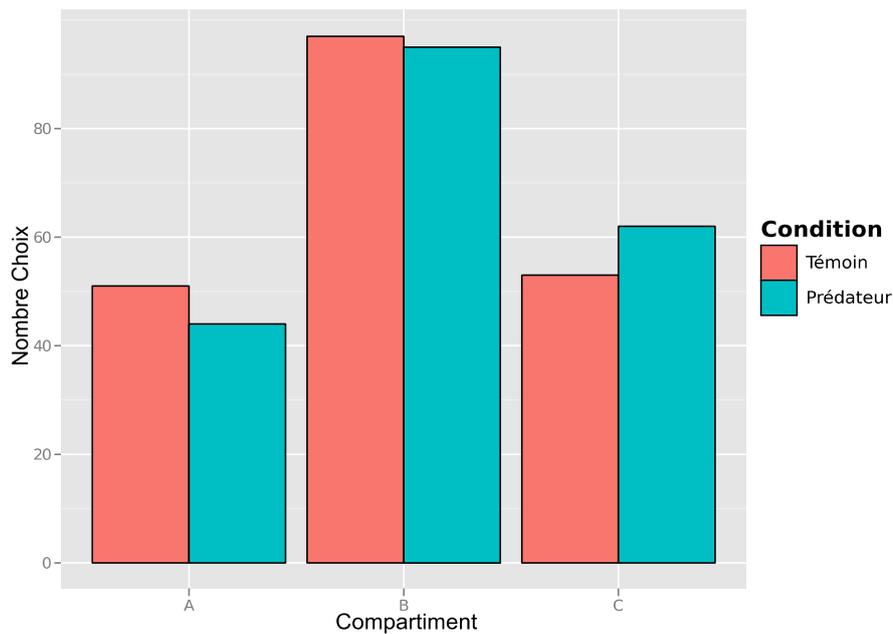


Figure 3.3: Distribution of fish larvae in the three compartments of aquarium in absence and presence of predators.

3d) Discussion

Chi-square conducted behavior' life trait showed that fish larvae life alone were not significantly attracted by conspecifics ($p = 0.112$), while fish larvae life in school were significantly attracted ($p = 0.004$). The solitary life trait of fish would accommodate for the fact that these species are not attracted to conspecifics. They are not motivated to choose conspecifics as they are not drawn to them, whereas schooling or couple fish have the tendency to be attracted to conspecifics as they are drawn to them. Their intuition motivates them to move in the direction of conspecifics when the opportunity is presented to them.

Studies have shown that schooling occurs prior to settlement (eg. Sancho *et al.* 1997, Leis & Carson Ewart 1998). There are species that school to some extent as reef associated juveniles, so schooling prior to settlement should not be a surprising. Settlement of a schooling larva would not be independent of the other individuals in the school (Breitburg *et al.* 1995). Schooling in larvae might also have the antipredator advantages that are normally attributed to schooling in adults, and could act to enhance the orientation of larvae (many sets of sense organs may be better than one) and, therefore, the ability of larvae to locate reefs and settle onto them. Schooling also increases their efficiency of foraging or finding food.

The chi-square conducted color' life trait showed that fish larvae with transparent body were not significantly attracted by conspecifics ($p = 0.588$), while fish larvae with juvenile color were significantly attracted ($p = 0.079$). Amongst its many functions coloration can also be used to help species with recognition of conspecifics. Therefore larvae with juvenile color were attracted to conspecifics as recognize the conspecifics with respect to their color.

Lastly, chi-square conducted swimming abilities showed that fish larvae with low swimming abilities were not significantly attracted by conspecifics ($p = 0.658$), while fish larvae with medium or fast swimming abilities were attracted ($p = 0.012$ and 0.020 , respectively). Recent evidence has suggested that some late stage larval fish have substantial swimming and sensory capabilities, which could allow them to influence their dispersal and subsequent recruitment (Stobutzki & Bellwood 1994, 1997, Leis *et al.* 1996, Leis & Carson- Ewart 1997). Larvae need to be able to orientate themselves to utilize their swimming capabilities for directed motion. Since larvae with medium and fast swimming abilities are able to orientate themselves they are able to swim in the direction of conspecifics when they are presented with this opportunity.

Thus, chi-square was conducted on each life trait and showed that fish larvae were not repulsed by the presence of predators (all p-value > 0.05). The presence of predators did not repulse the larvae because the larvae had adaptations that would enable them to evade the predators. Whether it be their social life, coloration or swimming abilities. The larvae were well adapted for the environment in which they were to spend their lives in.

4) Variation in Brain Organization in coral reef fish at larval stage

4a) Introduction

In coral reefs, while evidence is mounting that larval reef fish, cephalopod and crustaceans are active participants in the process of dispersal and recruitment, the sensory and behavioral mechanisms by which larvae disperse and return from their oceanic phase to appropriate recruitment habitat remains unknown (Montgomery *et al.* 2006, Leis *et al.* 2011). The answer must lie partly in the sensory abilities of marine species at larval stage. Indeed, without perceive the information emanating of environment which surrounds them, the habitat selection is inefficient (Myrberg & Fuiman 2002).

Perception can be defined as the active process of selecting, organizing and interpreting the information brought to the brain by the senses. The brain gathers and processes information it receives from the five senses (touching, seeing, feeling, tasting, and hearing). The cerebrum or telencephalon controls higher functions such as thought, memory, language and emotions, as well as voluntary movements. In reptiles, amphibians and birds, it becomes contains greater concentrations of integrative functions and in mammals, it is the major integrative part of the brain (Russell *et al.* 2008). The cerebellum is associated with sensory systems used for tracking movements of targets in the environment, as well as movements made by the animal itself, in all vertebrates, not just in a few isolated cases (Paulin 1993). The optic tectum (superior colliculus in mammals) in vertebrates has long been known to mediate such functions as visually identifying moving objects and orient toward attractive ones or escape if the object seems threatening (Schneider 1969, Ingle 1973). The medulla oblongata (vagal lobe) controls automatic functions such as breathing, digestion, heart and blood vessel function, swallowing and sneezing. Motor and sensory neurons travel through the medulla and it helps in the transferring of messages between various parts of the brain and the spinal cord (Russell *et al.* 2008). The Hypothalamus (hypothalamic lobe) (1) controls the release of some major hormones by the hypophysis and is involved in (2) temperature regulation, (3) control of food and water intake, (4) sexual behavior and reproduction, (5) control of daily cycles in physiological state and behavior, and (6) mediation of emotional responses (Hadley & Levine 2007, Coll *et al.* 2007).

There are large quantitative data sets on brain organization of vertebrate groups (Yopak 2007). Strong correlations have been found between brain patterns and various ecological factors, such as diet and feeding habits in teleosts (eg., Bauchot *et al.* 1977, Huber & Rylander 1992) and mammals (eg., Eisenberg & Wilson 1978, Pirlot & Jolicoeur 1982), habitat complexity in teleosts (Huber *et al.* 1997), birds (Riddell & Corl 1977), and mammals (Barton *et al.* 1995), and increased sociality and/or cognitive skills in birds (Lefebvre *et al.* 1998, 2002) and mammals (Kudo & Dunbar 2001). In contrast

to other vertebrate groups, there is a lack of quantitative information on brain organization and the relative development of major brain areas in coral reef fish at larval stage. The approximate mechanism (e.g., detection of cues and associated behavioral responses) underlying the recruitment process will partly shape growth and survival of individual until adult stage (Lecchini *et al.* 2007). Therefore, different parts of the brain could play different roles in the detection of cues and different function in processing the information received.

In this chapter, the structural diversity of coral reef fish brain at larval stage will be explored for the first time. Specifically, the present study explored the brain organization (telencephalon, optic tectum, cerebellum, vagal lobe and hypothalamic lobe) of fish captured at larval stage at Moorea Island, French Polynesia. The study was conducted on 25 species belonging to 11 fish families. For each species, the size of each part of the brain was recorded on 3 individuals.

4b) Materials and Methods

- Study Area & Specimen Collection

Fish larvae were captured with two crest nets (spaced out from 25 m) on the west coast of Moorea Island (17°31'7.38S, 149°55'20.89W). Catches were sorted and fishes identified using the guide provided by Maamaatuaiahutapu *et al.* (2006). The sampling was conducted daily from 07/03/2011 to 20/05/2011. Overall, 25 species were captured and 3 individuals of each species were used for the description of brain morphology.

- Brain Description

Once fish larvae captured, each individual was immediately put into a solution of MS 222. Once unconscious a picture of the larvae was taken, before been fixated in 10% formol for 24hrs. To get a solution of 10% formol, 37% formaldehyde was diluted with seawater using a ratio of 1:3 (formol:seawater). After 24hrs, the fish was removed and pictures were taken of the fish larvae without the cranium, dorsal view of the brain, ventral view of the brain, and two lateral views of brain. Pictures of the five main parts of the brain (Telencephalon, Optic tectum, Cerebellum, Vagal Lobe and Hypothalamic Lobe) were taken separately. The weight and length of fish, and length and width of head was measured before dissection. Upon removal of the brain, the weight, length and width of brain were measured. The five main parts of brain (Telencephalon, Optic tectum, Cerebellum, Vagal Lobe and Hypothalamic Lobe) were separated and the length of each part was taken. The five main parts of the brain of the fish larvae were fixated in 10% formal.

The length and width of body and head was measured to the nearest 0,01mm using a vernier caliper. The length and width of brain and of each brain part were measured using a scale on the

microscope and converted to the nearest 0.001mm. The pictures were taken using a camera which was connected to a computer, and using Sofortbild software the light intensity and contrast were adjusted before the picture was taken.

- Statistical Analysis

The data was entered into excel table and comparison of the five main parts of the brain with different parameters was made. Comparison was made amongst Family, Diet, Diel, and sensory abilities of the 25 species of coral reef fish larvae that was dissected. Graphs were drawn to make the comparison of the different brain parts and the different parameters.

4c) Results

The 25 coral reef fish species studied exhibited variation in both body length and brain length at larval stage. Of the total brain length, the telencephalon ranged between 18-29%, the optic tectum between 23-44%, the cerebellum between 7-36%, the vagal lobe between 7-17%, and the hypothalamic lobe between 12-23%.

Family

The 11 coral reef fish family studied exhibited variation in the five different parts of the brain length at larval stage (Fig 4.1). The smallest percentage with respect to length occupied by the Telencephalon belonged to the Fistularidae family (17%); Optic tectum belonged to the Acanthuridae family (24.6%); Cerebellum belonged to the Synodontidae family (7%); Vagal Lobe belonged to Fistularidae family (diminished vagal lobe); and Hypothalamic Lobe belonged to the Acanthuridae family (13.8%).

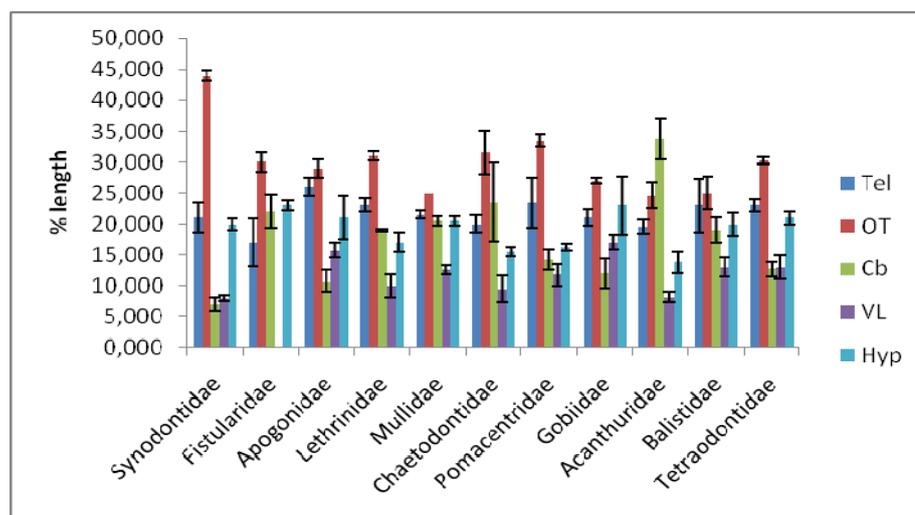


Figure 4.1: Variation of the five different parts of the brain for the 11 families dissected.

Diet

The 25 species dissected were distributed into three categories according to their diet (carnivores, herbivores and omnivores) (Fig. 4.2). The percentage of the mean length with respect to the total length of the brain of the five parts of the brain was calculated for the three categories (Fig 5.2). The largest Telencephalon was found in Omnivores (22%), largest Optic Tectum was in Carnivores and Omnivores (31%), the largest Cerebellum was in Herbivores (24%), the largest Vagal Lobe was in Carnivores (12%) and the largest Hypothalamic lobe was in Carnivores (20%).

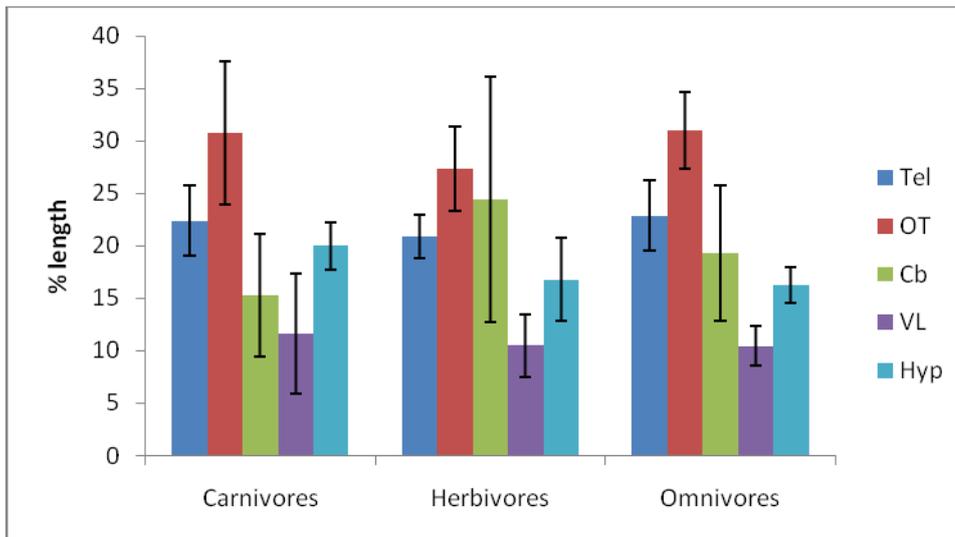


Figure 4.2: Variation in percentage of the mean length of the five main parts of the brain according to the diet of the 25 species of coral reef fish larvae dissected.

Diel

Species that were nocturnal were separated from those that were diurnal and the percentage of the mean length of the five main parts of the brain was compared (Fig. 4.3). The largest Telencephalon (25%), Optic tectum (33%) and Vagal Lobe (13%) was found in Nocturnal species. The largest Cerebellum (21%) was found in Diurnal species while the Hypothalamic lobe (18%) was approximately the same size for both nocturnal and diurnal species.

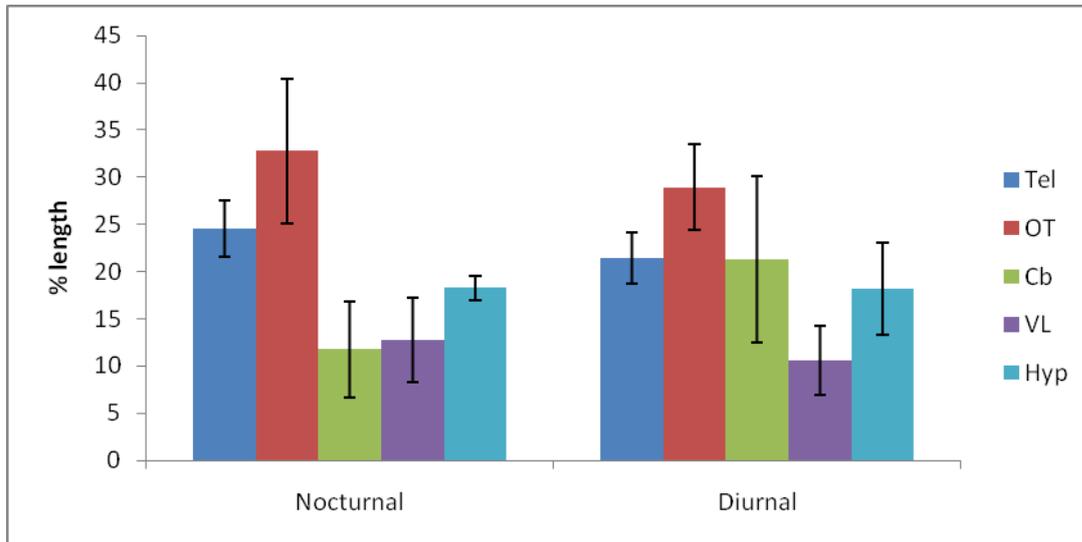


Figure 4.3: Variation in the percentage of the mean length of the five main parts of the brain for diurnal and nocturnal species.

Sensory Mechanisms

Upon comparing the percentage of the mean length of the five main parts of the brain according to the sensory mechanisms used for habitat selection (Fig. 4.4), the largest Tel (24%), OT (35%), VL (11%) was found in species that does not any of the three senses for habitat selection, the largest Hyp (18%) was found in species that uses chemical cues, the largest Cb (36%) was found in species that used mechanical cues.

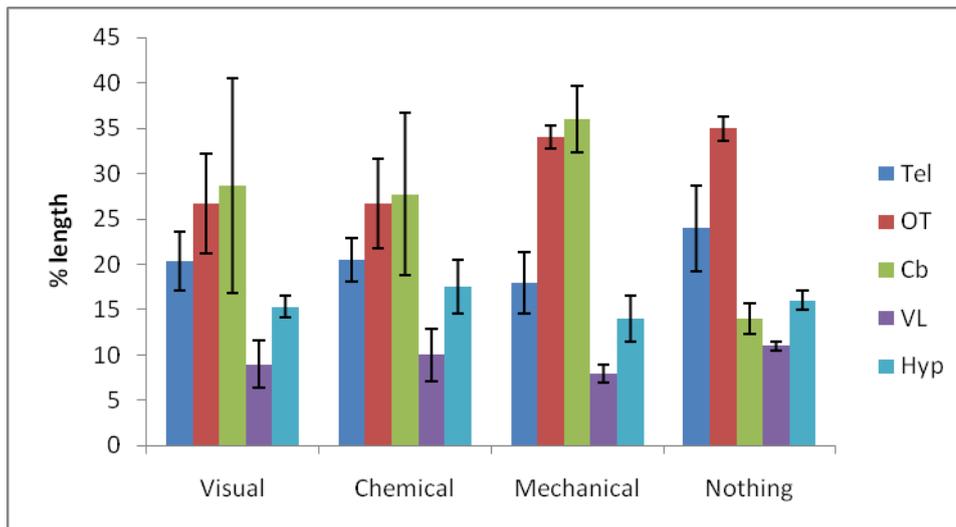


Figure 4.4: Variation in the percentage of the mean length of the five main parts of the brain according to the different sensory mechanisms used for habitat selection. With the species that does not use any of the other three sensory cues having the smallest of all five parts of the brain.

4d) Discussion

The order in which the fishes are represented is phylogenetically by family, beginning with families having characters considered to be primitive. Among these characters for the Antinopterygii are the low position of the pectoral fins on the side of the body, the origin of the pelvic fins in the posterior abdominal position, the lack of true spines in fins, and the maxilla bearing teeth and forming a prominent part of the gape.

Discovering and remembering the location of particular food patches and mapping their status and renewal rates, as well as identifying and remembering the predation risk associated with different locations, improve fish foraging efficiency and survival probabilities (Salas *et al.* 2008). Recent studies have shown that the teleost telencephalon contains essential components of the neural network that underlies map-like spatial memories in fish and therefore is necessary for specific spatial learning and memory functions (Rodriguez *et al.* 1994, Salas *et al.* 1996a, 1996b). Therefore, it is appropriate that the largest Tel belongs to omnivores as they have to both remember locations of food patches and identities of likely preys and predators. In vertebrates the optic tectum has long been known to mediate such functions as visually identifying moving objects and orient toward attractive ones or escape if the object seems threatening. It is a crucial center for sensorimotor for integration and for the generation of egocentrically referenced actions in space (Salas *et al.* 2008). This would help carnivores and omnivores distinguish prey from predators.

Nocturnal fish are active at night when there is little to no light available, for this they would have to acquire adaptations that would enable them to find their way around the reef and to be able to locate and spot potential prey. This study shows that nocturnal fish have the largest Tel, OT and VL, this is consistent as they need to have a good representation of their surrounding and Tel is responsible for map-like spatial memories (Rodriguez *et al.* 1994, Salas *et al.* 1996a,b) and OT enables the fish to visually identify moving objects as prey or predator. Visual information is relayed to the cerebellum by the pretectal nuclei, which project to the valvula cerebelli directly, not via the optic tectum. The teleost fish cerebellum is likely not only an essential center for motor coordination and adjustment but is also involved in learning and memory and in spatial cognition (Salas *et al.* 2008). Diurnal fish have the availability of light at their advantage and at the advantage of their prey as well there for agility and well coordinated movement on the part of the predator is necessary and for this a well developed cerebellum is essential for diurnal fish.

Cerebellum is associated with sensory systems used for tracking movements of target in the environment, as well as movements made by the animal itself, in all vertebrates not just in a few isolated cases. Therefore it is appropriate that fish which use mechanical cues for habitat selection have the largest cerebellum.

5) Relationship between visual abilities of coral reef fish larvae and their brain organization

5a) Introduction

Fish has been largely considered as the most primitive and least evolved vertebrate group. The ray-finned fishes represent the largest and most diverse vertebrate radiation, with an enormous range of variation in brain and behavior complexity and specific adaptations (Broglia *et al.* 2003). As teleost fish and amniotes share a common ancestor similarities in brain and behavior organization could reflect the traits of an ancestral pattern of organization, conserved along evolution and through a vast repertoire of marvelous adaptations to different habitats and modes of life (Broglia *et al.* 2003). Fish use diverse sources of spatial information, such as self-generated movement cues from different sensory modalities, directional cues that polarize the environment or positional landmarks that enable inferring the place locations relative to distances, angles and the geometry of objects within an array (Burgess *et al.* 1999, Jacobs 2003).

The forebrain of vertebrates shows an impressive range of morphological variation and specialized adaptations (Nieuwenhuys *et al.* 1998, Northcutt 1995). For example, whereas in amniotes and other non-actinopterygian groups the telencephalon develops by a process of evagination, in the actinopterygian fish, the telencephalon undergoes a process of eversion or outward bending during the embryonic development (Nieuwenhuys 1963, Nieuwenhuys & Meek 1990, Northcutt & Bradford 1980). The cerebrum or telencephalon controls higher functions such as thought, memory, language and emotions, as well as voluntary movements. There are data that indicates that the teleost telencephalon (or some areas of this brain region) has specific functions in spatial learning and memory, providing a neural substratum for cognitive mapping in fish (Rodriguez *et al.* 1994, Salas *et al.* 1996a,b).

The teleost optic tectum is a crucial center for the generation of egocentrically referenced actions in space and for sensorimotor integration. It represents a spatially ordered motor map in the deep tectal layers in correspondence with the retinotopic visual map in the superficial layers (Salas *et al.* 1997, Sparks 2002). Vertebrates are able to visually identify moving objects and orient toward attractive ones or escape if the object seems threatening. The optic tectum (superior colliculus in mammals) (OT/SC) has long been known to mediate such functions (Schneider 1969, Ingle 1973).

5b) *Methodology*

- Brain Organization

Once fish larvae were captured with crest nets, three individuals of each species were immediately put into a solution of MS 222. Once unconscious, a picture of the larvae was taken. After which it was fixated in 10% formol for 24hrs. To get a solution of 10% formol, 37% formaldehyde was diluted with seawater using a ratio of 1:3 (formol:seawater). After 24hrs, the fish was removed and pictures were taken of the fish larvae without the cranium, dorsal, ventral and two lateral views of brain. Pictures of the five main parts of the brain (Telencephalon, Optic tectum, Cerebellum, Vagal Lobe and Hypothalamic Lobe) were taken separately. The weight and length of fish, and length and width of head was measured before dissection. Upon removal of the brain, the weight, length and width of brain were measured. The five main parts of brain (Telencephalon, Optic tectum, Cerebellum, Vagal Lobe and Hypothalamic Lobe) were separated and the length and width of each part was taken. The five main parts of the brain of the fish larvae were fixated in 10% formal. The weight of body and of brain was measured to the nearest 0.001g using a balance. The length and width of body and head was measured to the nearest 0,01mm using a vernier caliper. The length and width of brain and of each brain part were measured using a scale on the microscope and converted to the nearest 0.001mm. The pictures were taken using a camera which was connected to a computer, and using Sofortbild software the light intensity and contrast were adjusted before the picture was taken.

- Telencephalon Lesion

The telencephalon was removed by aspirations according to the methods described by Salas *et al.* (1996a, b) and Lopez *et al.* (200a, b). The fish were anesthetized with tricaine methanesulfonate (MS 222) (0.1g/L of seawater), and then immobilized in a surgical chamber with constant flow of aerated water through the gills. The concentration of anesthetic in the water was kept at 0.1g/L during the surgery. The dorsal skin, mussel, and skull were removed carefully under visual control by means of a binocular microscope, and the fatty tissue present below the skull was removed by aspiration to expose the brain. The Telencephalon was identified and the lesion was performed by means of a glass micropipette connected to a manual vacuum system (a 60ml syringe). After ablation, the piece of skull was replaced to its original position, and fixed with cyanoacrylate glue, and covered with scales from the fish. Animals were put in a separate tank and allowed to recover.

5c) Results

- Brain Organization

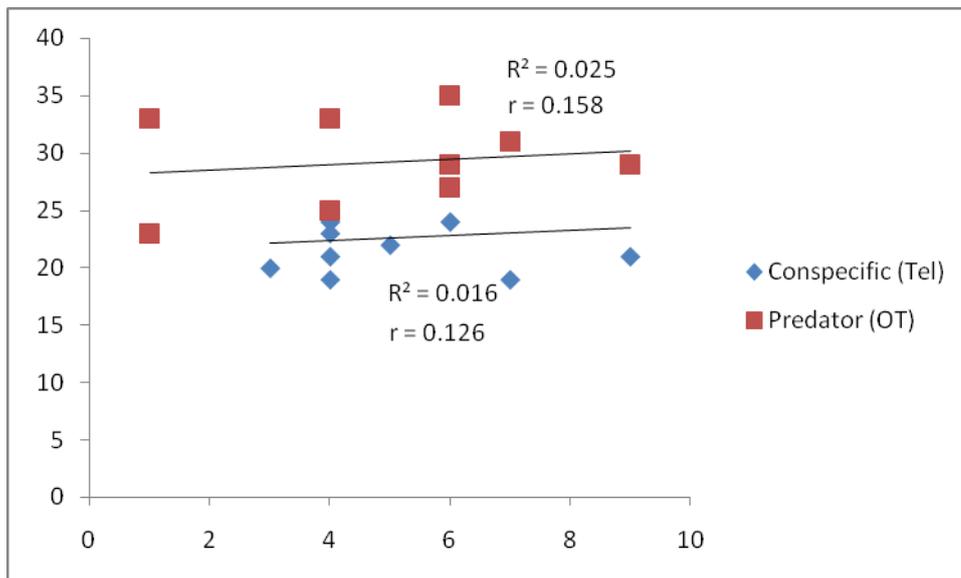


Figure 5.1: Percentage of the length of the Tel and OT of coral reef fish larvae against the number of fish that is attracted to conspecifics or predators.

The percentage of the length of the Tel and OT of coral reef fish larvae against the number of fish that is attracted to conspecifics or predators does not show significant correlation ($r = 0.158$ and $r = 0.126$ respectively).

- Telencephalon Lesion

The lesion technique was successfully learned due to the help of Emilio Duran from Sevilla University. The success was determined by the survival of the fish after surgery. The lesions were conducted on adult fish. This study is still ongoing with more tests to be done. All surgical equipment will be modified to suite larvae as the main aim of this project is to work on coral reef fish larvae. Surgery and test on coral reef fish larvae will be conducted on a later date.



Fig 5.2. The setup that is used for the surgery of the fish.



Fig 5.3. Surgery in progress. The removal of the cranium using simple surgical tools.



Fig 5.4. The cranium has been removed thus exposing the telencephalon of the Mullidae.



Fig 5.5. The removal of the telencephalon by a process of aspiration.



Fig 5.6. Successful surgery. The fish is released into the tank for recovery.

5d) *Perspectives in 2012*

The brain morphology of coral reef fish larvae were found to vary among species. This could be due to different life traits among families and species. Different species also use different sensory mechanism for settlement selection which could also account for the difference in shape and size of the different brain parts in coral reef fish larvae. The lesion technique was a success but needs to be improved. This study will be continued in 2012 with a trip to Seville to learn more of the technique and to study the brain morphology of coral reef fish larvae. This study will enable me to distinguish the different parts of the brain better whereby I will be better understand the brain of coral reef fish larvae and will be able to concentrate on specific areas in the brain. The surgery equipment will be modified to suit larvae. Although the technique was conducted on adult coral reef fish, the main aim of this study is to work on coral reef fish larvae and to try and understand how the brain of coral reef fish perceives information with respect to habitat selection.

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