Factors influencing the development and mortality rate of shy and cat shark embryos in South African waters

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The work contained in this thesis is original, except where otherwise indicated either in the text, or in the list of acknowledgements.

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Abstract

There are at least 500 species of sharks inhabiting the world's oceans and very little is known about the reproductive biology of most of these species. By observing Scyliorhinids, which exhibit oviparous reproductive behaviour, we can further understand the nature of elasmobranch reproductive behaviour, including embryo growth and development. Two Scyliorhinid species endemic to South African waters are investigated here, *Poroderma pantherinum*, commonly known as the Leopard catshark and *Haploblepharus pictus* the Dark shyshark. Observations were made both in the field and in the laboratory to ascertain differences in reproductive behaviour. In the field *P. pantherinum* laid their eggs at an average depth of 15.7 m ± 28.8 and their preferred choice of substratum, onto which their eggs were singly attached, were seafans. *H. pictus,* on the other hand, laid their eggs at an average depth of 3.9 m ± 15.9 and their preferred choice of substratum, onto which they attach their eggs predominantly in pairs, was the seaweed *Bifurcariopis capensis.* The average temperature at which the eggs were found were similar for both species (15°C) and eggs were observed to have been laid all year round.

Observations of embryo development were made in the laboratory under different sets of controlled environmental conditions. The two temperatures to which the eggs were exposed were 14°C and 17°C and the condition of the water was either turbulent or still. Embryo length-age relationships were linear for both species. There was no significant difference in growth rate under turbulent or still conditions. There was, however, a significant difference in growth rate between embryos exposed to different temperatures. For *P. pantherinum* the average growth rate in 14°C turbulent conditions was 0.048 cm/day ± 0.0001 and 0.05 cm/day ± 0.0002 in 14°C still conditions. In 17°C in turbulent conditions it was 0.06 cm/day ± 0.0005 and 0.07 cm/day ± 0.0001 in 17° still conditions. The average incubation period for *P. pantherinum* was recorded to be 39 weeks ± 1.5 at 14 °C and 25 weeks at 17°C. The embryo grew 1.4 times faster in a temperature raised by 3°C. For *H.*

pictus, the average growth rate in 14°C turbulent conditions was 0.04 cm/day ± 0.0003 and 0.05 cm/day ± 0.0001 in 14°C still conditions. In 17°C turbulent conditions, the average growth rate was 0.09 cm/day ± 0.001 and 0.07 cm/day ± 0.001 in 17°C still conditions. The incubation period was recorded to be 30.7 weeks ± 7.25 at a temperature of 14°C and 27.5 ± 1.9 at a temperature of 17°C. The embryo grew 1.8 times faster in a temperature raised by 3°C.

The morphological changes taking place at different stages during development were recorded, including appearance of external gill filaments, opening of hatching slits, development of mouth and gills and absorption of external gill filaments. The change in yolk weight versus body weight was also calculated at different stages of development. The results indicate that an increase in body mass is directly proportional to a decrease in yolk mass. Development in the laboratory was also compared to that in the field. The results showed no difference in development of embryos exposed to a temperature of 14°C as to those in the field. Temperature recordings reveal that there were greater fluctuations in temperature, where *P. pantherinum* egg cases were found at >10 m as opposed to shallow waters, where *H. pictus* eggs were found.

To determine whether *P. pantherinum* and *H. pictus* egg cases possess anti-fouling properties, live eggs, empty eggs and plastic panels were placed in the ocean at the depths at which eggs were naturally laid. The replicates were then harvested at intervals of three, five and seven months and the percent fouling cover on the different egg cases were compared. There was no significant difference in percentage cover of species between the different egg cases, however, there was a significant difference in percentage cover over time i.e. the longer the eggs were exposed to environmental conditions in the ocean, the more growth was observed on the egg cases. Different species colonized the egg cases, according to the depths at which they were placed. This indicates that light and temperature influence the settlement of different epibionts and there is some evidence suggesting that

the embryo/egg case has the ability to deter fouling for at least five months during development.

Chapter 1

Introduction

Cartilaginous fishes fall within the Class Chondrichthyes, which includes all the sharks, rays and chimaeras. There are 10 Orders within this Class, which are grouped into two Subclasses—Elasmobranchii and Holocephali. Sharks fall within the subclass Elasmobranchii, representative of all the sharks, skates and rays, while Subclass Holocephali comprises the elephantfish and chimaeras. Eight extant orders are found within elasmobranchs, with 500 species recorded thus far (Compagno, 2005). Sharks of the order Carcharhiniformes have the highest number of extant species and are commonly known as ground sharks. There are 225 species recorded presently and these are divided into eight families. Scyliorhinidae is the largest family in the Carcharhiniformes, comprising 160 species, with more new species continually being discovered. However, the diversity among these species is limited. Scyliorhinids comprise 46% of the carcharhinoid species, but only 30% of the genera, whereas Carcharhinidaes, for example, comprise 20% of both the genera and species, indicating greater diversity. According to White (1937) the phylogeny of scyliorhinids suggests that they are the most primitive of the Carcharhiniformes due to the position of their dorsal fin and reduced vertebral calcifications. They are found in shallow intertidal areas down to depths of 2000 m (Compagno, 1984).

Reproduction in elasmobranchs

Wourms (1981) made the distinction between two major forms of viviparous reproduction in elasmobranchs - lecithotrophy (where embryo development is completely dependant on the yolk), and matrotrophy (where there is some dependence on the mother for an additional food source). The latter was then further subdivided into oviparity (where the embryo develops outside the mother's body), yolksac viviparity (where the embryo develops in an egg case within the uterus) and aplacental viviparity (internal development). Oviparity can be further subdivided into single (external) and multiple (retained) (Nakaya, 1975; Compagno, 1990). Elasmobranchs exhibit all the modes of reproduction inherent in vertebrates (Dulvy and Reynolds, 1997), but according to Compagno (1988) the most common modes of reproduction among sharks are ovoviviparity and viviparity. Figure 1.1 illustrates the percentage of species, in the Carcharhiniformes, that exhibit oviparity, viviparity, or ovoviviparity (Compagno, 1988). Viviparity seems to be the most common form of reproduction within this order, with just under 50% of the species represented, followed by oviparity with 33%. Oviparity only occurs in a few sharks, mainly bullhead sharks of the Order Heterodontiformes, some Orectolobiformes, Proscylliidae and Scyliorhinidae (Compagno, 1988). Table 1 highlights the families within this order and their mode of reproduction with which they are associated. This clearly indicates that egg-layers and live-bearers are present and that two modes of reproduction may be occur within the same family. For example, species in the Family Triakidae are either viviparous or ovoviparous with the maximum number of offspring recorded from a species being 38. The maximum gestation period observed is 15 months. Another example of a family exhibiting two modes of reproduction is Proscylliidae, which also exhibit either ovoviviparity or viviparity. The maximum number of offspring recorded by a species in this family is only two, with an unknown gestation period. Both live-bearing and egg-laying species have embryos that have similar nutritional needs that are met in different ways i.e yolk, uterine milk, oophagy (intrauterine cannibalism where the hatching embryo eats the other eggs), adelphophagy (intrauterine cannibalism where the developing embryo eats the other embryos), or placental nutrition. Researchers believe that producing live young is advantageous, due to an increase in survival of offspring, even though fewer young are produced (Shine, 1989; Clutton Brock, 1991). Ovoviparous and viviparous species tend to have a lower fecundity than oviparous species. Table 1.1 indicates that egg producing Scyliorhinidae are able to lay up to 60 eggs annually (Musick and Ellis, 2005), whereas live-bearing Proscylliidae produce a maximum of two young.

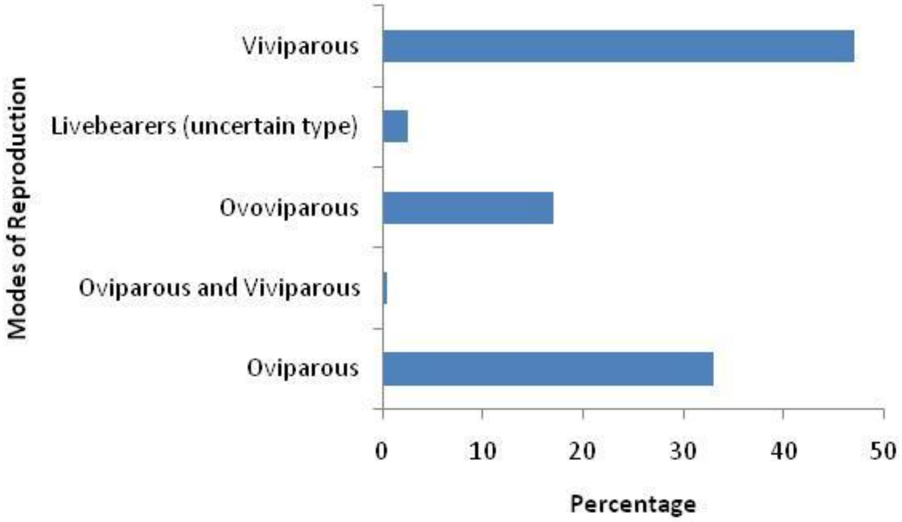

Figure 1.1: Percentage breakdown of the families within the Carcharhiniformes exhibiting the various modes of reproduction (Compagno, 1988).

Table 1.1: Reproductive biology of the families within the Carcharhiniformes (Compagno *et al*, 2005).

Family	**Mode of reproduction**	**Max. number of offspring**	**Max. gestation period (months)**
Scyliorhinidae	oviparous	60	>12
Triakidae	ovoviparous/viviporous	38	15
Hemigaleidae	viviparous	19	7
Carcharinidae	viviparous	24	12
Sphyrinidae	viviparous	43	11
Proscylliidae	ovoviparous/oviparous	2	unknown
Pseudotriakidae	ovoviparous	4	unknown
Leptochariidae	viviparous	7	4

Bet Hedging

The theory of Bet Hedging was introduced in order to explain how female individuals should increase their level of fitness in an environment that is uncertain. The idea is to lower the fitness variance between years in order to maximize the long- term fitness (Slatkin, 1974; Seger and Brockman, 1987; Philippi and Seger, 1989). In 1973, Holden made the assumption that there were more advantages to being an egg-layer, as opposed to being a live-bearer. One of the advantages of laying eggs is that egg-layers had a higher fecundity (ability to produce more offspring), due to independence of uterine size and shorter gestation periods. The only limiting factor was the rate of egg deposition. Eggs that escape predation could then result in a higher number of neonates reaching maturation. However, live-bearers are better protected at the embryonic stage and the neonates are bigger and have fewer natural predators. Since small sharks are more vulnerable to a wider variety of predators, such as large teleosts, birds and marine mammals, this could result in greater survival, despite lower fecundity. Large species like the ovoviparous tiger shark (*Galeocerdo cuvieri*) can produce a litter of 82 embryos, while the viviparous blue shark (*Prionace glauca)* can produce at least 135 pups. This is a higher level of fecundity than many small, egg-laying Scyliorhinidae species achieve. However, Bet Hedging describes an advantage to being a small, oviparous Scyliorhinid, as opposed to being a small viviparous species (Stearns, 1992). Smaller species, although they have a higher predation rate, have a high evolutionary fitness. For example, a pregnant viviparous species will have an evolutionary fitness of zero once it gets eaten, whereas an oviparous species that has already laid its eggs will have a better evolutionary fitness (due to embryos still surviving to develop outside the uterus).

Egg case development

Egg cases of Scyliorhinidae and Proscylliidae catsharks are depressed, subrectangular and elongated, with rounded undulated sides, a truncated concave or convex anterior or vestibular end (through which the hatching shark escapes), a narrow posterior terminal end, usually a pair of short to long horns with tendrils at both ends, and generally a constriction on the capsule near the vestibular end (Compagno, 1988). Cases are made up of keratin-like collagen (Brown, 1975).

Past research has provided insight into the characteristics of egg cases of scyliorhinid and proscylliid catsharks to ensure optimum development and survival. Smith *et al*. (1967) documented that the egg case of *Raja erinacea* has the ability to produce an osmotically isolated environment until the embryo is capable of urea retention and osmoregulation. According to Rodda and Seymour (2008), the osmotic concentration of the egg jelly and sea water are primarily comprised of chloride (almost half) indicating that Sodium Chloride is the main constituent responsible for osmoregulation (Table 1.2). The yolk, however, has a very small Chloride component, with the rest being made up of organic compounds, urea and trimethylamine oxide (TMAO), which are crucial osmoregulatory components for elasmobranchs.

Table 1.2. Osmotic concentration of jelly, seawater and yolk (%) (Rodda and Seymour, 2008).

	Jelly	Seawater	Yolk
Chlorine	48	47	14
Sodium Chloride	52	53	28
Organic compounds	0	0	58

Eggs that are laid with tendrils are soft and pliable at oviposition and harden with continual exposure to sea water (Flammang, 2005). Oviposition has been observed in aquaria, as well as in the wild with the help of an ROV. Video footage was captured at a depth of 300-400 m of two species of catsharks (*Apristurus brunneus* and *Parmaturus xaniurus*) entangling egg cases on stationary substrata at a temperature of 6°C (Flamming, 2005). In captivity, the female was observed swimming around a vertical object while the anterior tendrils of the egg case wrapped themselves around the object (Springer, 1979; Castro *et al.,* 1988). *Heterodontus francisci* and *H. portusjacksoni* use their mouth to place their eggs in rocks and crevices, because there are no tendrils to wrap around the substratum (Flammang, 2005; Powter and Gladstone, 2008). This, however, can allow the eggs to be washed away, because they are not securely anchored to the ocean floor. Lack of tendrils also has implications for the developing embryo, as the ventilation fissures could block as a result of the egg case lying directly on the ocean floor. The incoming flow of seawater would also be limited due to the poor positioning on the sea floor (as opposed to egg capsules with tendrils that can "hang" off the substratum). Limited passive ventilation would require active ventilation by the embryo, which would have metabolic implications for the embryo. Active ventilation by *Apristurus* has been observed by Flammang (2005), where the embryo uses it tail to sweep water in through the open fissures to allow for greater oxygen exchange. Embryos encased in egg cases with long tendrils were less active, as they were already exposed to passive ventilation, which is as a result of pressure differences between the internal and external environment that causes a "pitot tube effect" and a "venturi effect" due to the position of the upstream and downstream fissures.

Scyliorhinidae eggs have been described by Ebert *et al*. (2006) as having a pair of respiratory fissures at both the anterior and posterior ends of the egg case. The fissures are closed by albumen during the first few months of embryo development. The time it takes for the albumen to dissolve varies amongst species, with *Scyliorhinus canicula* taking *c*70-80 days to open (Ballard *et al*., 1993), and *Heterodontus portusjacksoni* *c*120 days (Rodda and Seymour, 2008). The fissures are then completely open to allow the entry of oxygenated water and to remove metabolic wastes. Some water, however, enters the egg case through osmotic diffusion, but not sufficient to assist in waste exchange, which only occurs at 5% the rate of osmotic diffusion (Hornsey, 1978; Koob and Cox, 1993, Thomason *et al.,* 1996). The embryo possesses a hatching gland which, upon secretion, dissolves the albumen in the fissures for ventilation, and that in the hatching slit once the embryo is ready for hatching (Ballard *et al.*, 1993). Hatching takes place when the food reserves (external yolk sac) are depleted.

Family Scyliorhinidae

There are at least 152 known catshark species and very little is known about their reproductive biology. The only information recorded thus far has been observations of viviparity and oviparity, single and multiple oviparity and egg laying rates. Castro *et al. (*1988) observed *Scyliorhinus rotifer* in captivity and deduced that eggs are deposited every 14-16 days. Further research has revealed that even though catsharks lay eggs all year round, there are certain times of the year when oviposition is more common (Chen *et al.,* 1996; Ellis and Shackley, 1997; Richardson *et al.,* 2000). This has further been substantiated by Ebert *et al.* (2006), who determined that species such as *Apristurus microps* produce eggs mostly in summer and winter, *Scyliorhinus capensis* mostly in winter and *Holohalaurus regani* all year round*.* There is also a distinct spatial separation according to size, maturity and maturity stage in deepwater chondrichthyans and it is believed that once the gravid females have deposited their eggs, they return to deeper waters (Kyne and Simpfendorfer, 2007). Castro (1993) discovered that Bulls Bay*,* off the coast of South Carolina USA, has distinctive nursery areas where several shark Carcharhiniform species give birth (i.e. Spinner *C. breuipinna*, Sandbar *C. plumbeus*, Dusky *C. obscurus*) and the Smooth Dogfish *Mustelis canis* that lays eggs. These locations, which are mainly situated in shallow water, have high productivity where food is easily accessible for juveniles. Springer (1967) observed catshark species in the Atlantic Ocean and recorded females to be more abundant than males in such nurseries.

Catsharks in Southern Africa

Southern Africa is home to a rich diversity of sharks with at least 46 families, 106 genera and 210 species thus far recorded (Ebert *et al.,* 2006). There are 16 catshark species endemic to South African waters (Table 1.3). This makes up 8.6% of the known shark species recorded to date. Despite their abundance, there is very little information on their biology. Most of the species are oviparous, with the exception of *Halaelurus lutarius* and *Pseudotriakis microdon* that exhibit aplacental viviparity (Bass *et al*., 1975). The largest egg case recorded thus far has been from *Cephaloscyllium sufflans* and the smallest from *Holohalaelurus punctatus*. Not much information is known to date on the incubation period for most of the catshark species in Southern Africa, but for those which it is known, it seems to vary greatly, from 23 to 164 days (Bass *et al*., 1975). The size of the embryos at hatching for all species seems to fall within the same range (8-15 cm), except for that of *Pseudotriakis microdon,* which attains a size of 90 cm.

Table 1.3. Catshark species endemic to Southern African waters and their reproductive mode and statistics (Bass *et al.,* 1975; Ebert *et al*., 2006).

Species	**Reproductive mode**	**Size of embryo at birth (cm)**	**Incubation time (days)**	**Egg dimensions (cm)**
Apristurus microps	oviparous			
Apristurus saldhana	oviparous			
Galeus polli	aplacental viviparity			
Cephaloscyllium sufflans	oviparous			12 x 5
Halaelurus lutarius	ovoviparous	11		
Halaelurus natalensis	oviparous			4 x 1.5
Halaelurus lineatus	oviparous	8	23 -- 36	4 x 2
Haploblepharus pictus	oviparous	11	104	6 x 3
Haploblepharus fuscus	unknown	10 -- 15		
Haploblepharus edwardsii	oviparous	10		5 x 3
Holohalaelurus punctatus	oviparous			1.7 x 1
Holohalaelurus regani	oviparous			3.5 x 1.5
Poroderma africanum	oviparous	14	164	5 x 10
Poroderma pantherinum	not recorded			
Scyliorhinus capensis	oviparous			8 x 3
Pseudotriakis microdon	ovoviparous	90		

The two endemic catshark species under investigation in this study are *Haploblepharus pictus* and *Poroderma pantherinum.* Due to the abundance of *H. edwardsii* and *P. africanum* egg cases in the field, it was decided to include these observations for interest purposes. Compagno *et al*. (2005) reported that *P. pantherinum* is found on the continental shelf to upper slope and in the intertidal area down to 282 m. The substratum on which they are found is mostly rocky where there are caves in which they can hide. They are more active at night and feed on bony fish, hagfish, other small sharks, shark egg cases, and a wide variety of invertebrates. Compagno *et al.* (2005) reported that an egg case in captivity had an incubation period of 5.5 months. Smith and Griffiths (1997) described the egg case of *P. pantherinum* as being light brown to greenish brown in colour with thin tendrils and walls and 7 x 3 cm in dimension. The egg cases of *P. africanum* are more rectangular, robust and dark brown in colour, with dimensions of 9.5 x 4.5 cm. This species is found from the intertidal zone down to 100 m. *H. edwardsii* are also found on the continental shelf on sandy and rocky substrata. They occur to depths of 288 m, but are more commonly found between 30-90 m. Their diet consists of bony fishes, fish offal, crustaceans, cephalopods and polychaetes. *H. pictus* are found in the intertidal down to 35 m in kelp, rocky and sandy habitats. Their diet consists of bony fishes, sea snails, cephalopods, crustaceans, polychaetes, echinoderms and algae (Compagno *et al*., 2005). Egg development in captivity was observed to be 3.5 months (Compagno *et al*., 2005). The morphology of the egg case was described by Smith and Griffiths (1997) as being purse shaped with a variation in colour from amber to dark brown. The walls and tendrils are also much thinner than those of the *Poroderma* species. The eggs in this study all possess tendrils, which would be described as being “brunneus like” if they were found in the genus Apristurus.

The embryos of marine fishes are particularly sensitive during the process of gastrulation (early phase of embryonic development), due to the ability of temperature to affect enzymes that increase the metabolic rate (Lillie and Knowlton, 1897). There is a temperature range at which enzymes function optimally and should the temperature fall slightly out of the range it could lead to a decrease in the functionality of the enzyme process, resulting in embryo death. Laurence and Rogers (1976) who did a comparative study on Atlantic Cod *Gadus morhua* and Haddock *Melanogrammus aeglefinus* came to the conclusion that temperature (as well as salinity) is the main environmental variable determining embryonic growth and survival.

Temperature and embryo development

Although stages of embryonic development have been recorded in certain ovoviviparous shark species caught opportunistically through fishing, such as the Sandtiger shark *Carcharias taurus* (Gilmore *et al.,* 1983) and the Pacific Angel shark *Squatina californica (*Natanson and Calliet, 1986) there are few observations of developing embryos under controlled constant temperatures. First observations on developing shark embryos under controlled conditions were made by Smith (1942), who documented the development of the Japanese shark *Heterodontus japonicas*. Ballard (1993) made observations on *Scyliorhinus* embryos at a temperature of 16°C and was able to supply qualitative data on embryo development. He also attributed stages, but focused mainly on the prehatching phase of development (first three months). He gave a total of 34 stages for the embryo development of *S. canicula* with only a few embryo measurements*.*

Harris (1952) studied the rate of development of *S. canicula* and did a comparative study based on observations at two different temperatures. He determined that the rate of development was 1.64 times longer at a cooler temperature of 11.5°C as opposed to a warmer temperature of 15.5°C. From this information he was able to deduce that there were difficulties in determining the incubation period for oviparous species due to changes in temperature and exact date of oviposition.

Not much further information was gathered on the morphological characteristics of embryo development until Rodda and Seymour (2008) used *Heterodontus* eggs in order to assign stages to the developing embryos. This is a useful tool to assist future research being undertaken on embryo development to compare any differences. They assigned a total of 15 stages to the developing embryo at a temperature of 20°C to produce a staging table (Table

1.4). They also made a comparative study based on embryo development at different temperatures (18, 20 and 22°C) and concluded that temperature greatly affected the incubation period. It took an additional 90 days for the embryo to reach full development at a temperature of 18°C, as opposed to 22°C at which development took 291 days. Temperature seemed to have no effect on the timing of the opening of the mucous plugs.

Table 1.4. Rate of embryo development for *Heterodontus portusjacksoni* at 20°C and its assigned stages (Rodda and Seymour, 2008).

Days	**Length at 20°C (cm)**	**Stage and description**
15	0	**1-2:** gastrula and blastoderm observed
40	0.5	**3**: cervical and cephalic flexures
45	0.5 -- 1	**4**: otic and optic vesicles and branchial gill
60	1 -- 1.5	**5**: spiracular gill slit present, heart region active, physical embryonic activity
90	1.5 --2	**6**: yolk vascular, pectoral and dorsal fins shaped, yolk stalk becomes a distinct connection
100	2 -- 3	**7**: external gill filaments present
105	2.5 -- 4	**8**: caudal fin shaped, eye pigmentation begins
113	3 -- 4	**9**: mouth recognizable, dorsal fins narrow at base, fin rays appear in all paired fins, dorsal spine present
120	4 -- 5.5	**10**: capsule opens, dorsal spines and claspers
135	8 -- 9	**11**: external gill filaments maximum length (12mm), filament reabsorption begins, internal gills functional
150	12 -- 13	**12**: external filaments absent, body pigmentation starts, internal yolk develops
180	16 -- 17	**13**: body pigmentation complete
225	17 -- 19	**14**: external yolk absent
315	18 -- 22	**15**: internal yolk absent, hatching occurs

The first research undertaken on the development of catshark embryos in South Africa was by Von Bonde (1945a), who described the male and female reproductive organs and observed the development of two *Haploblepharus pictus* embryos that were laid in captivity. At the time, *H. pictus* was synonomous with *H. edwardsii (*Compagno, 1988) and he referred to the species as *H. edwardsii,* when in actual fact he was looking at *H. pictus.*

One egg remained in the aquarium to simulate the natural environment, so that the incubation period could be recorded, whilst the other embryo was completely removed from the egg case and observations on development documented. Von Bonde paid close attention to the respiration of the embryo and noted that external gills develop at an early stage of development. He determined that the main function for these appendages was for respiration. Rodda (2000) went on to determine that, due to the low oxygen levels in the jelly that surrounds the embryo, other respiratory aids are required. She pointed out that vascularization of the yolk sac, fin budding and external gill filaments assist the embryo at 50% saturation (80 Torr). They contain a high concentration of blood vessels with which to supply the embryo with sufficient oxygen. The gill filaments, however, have a dual purpose, in that they also play a role in urea retention until the embryo is capable of osmoregulation (Smith, 1936; Read, 1968, Kormanick, 1993). Fifty days into development they disappear at a stage called the “yolk sac stage” (Von Bonde, 1945a). It is at this stage that the internal gills become fully developed and operational and the yolk sac has been depleted to half its original mass. Von Bonde determined the incubation period for *H. pictus* to be 104 days and hatching took place once the yolk sac had been completely absorbed. Von Bonde (1945b) also went on to describe the intratuterine development of the ovoviparous species *Squalus ancanthis* and collected eggs from gravid females at different developmental stages. The vitelline circulation and external gills were again the main focus, with a detailed description on the primary stages of development.

Further research on catshark eggs in Southern Africa was undertaken by Smith and Griffiths (1997) who tried to determine the rate of hatching success of *Poroderma* and *Haploblepharus* species based on the proportions of beach cast egg cases that showed evidence of successful hatching. They estimated that *H. pictus* had a high rate of hatching success (61.7%) with low depredation rates (20.8%), as opposed to *P. pantherinum* that had a lower hatching success of 47% with high depredation rates.

Oxygen and embryo development

Rodda (2000) studied the rate of development of the embryos of Port Jackson sharks at different temperatures and recorded the oxygen consumption. The embryos grew exponentially until they slowed down near hatching. There was also a decrease in the weight-specific rate of oxygen consumption as the embryos grew bigger (The Q10 value for oxygen consumption decreased from 7.9 at 5 g to 0.9 at 55 g). The incubation period increased with decreased temperature, thus increasing the total lifetime oxygen consumption of the embryos.

Research undertaken on Bamboo sharks *Chiloscyllium plagiosum* at a temperature of 25°C also indicated that oxygen consumption increases exponentially, up until a certain age, where it then decreases linearly (Tullis and Peterson, 2000) (Figure 1.2). Spotted dogfish embryos *Scyliorhinus sp.*, however, showed an exponential increase in oxygen consumption right up until hatching (Diez and Davenport, 1987), similar to *H. portusjacksoni.* However, due to the paucity of data on metabolism and growth of sharks, it is difficult to determine the commonality. This does, however, prove that oviparous sharks, like oviparous reptiles, exhibit at least two metabolic patterns during embryonic development.

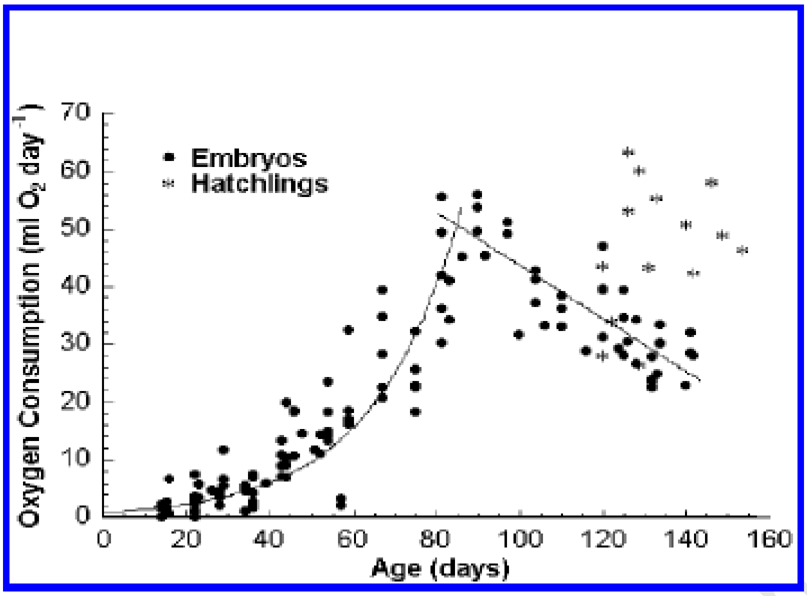

Figure 1.2: Oxygen consumption of *Chiloscyllium plagiosum* embryos (Tullis and Peterson, 2000. pp 275).

It has been hypothesized that changes in metabolism and growth rate in embryonic development are “causally interrelated (Vleck *et al.,* 1979; 1980; Vleck and Vleck, 1987). Leonard *et al,* (1999) were able to prove that embryonic movements from skates (*Raja erinacea*), which were used for ventilation purposes, increased the standard metabolic rate by approximately 20%. When determining the change in body mass with embryo development, Tullis and Peterson (2000) discovered that the body mass of Bamboo sharks increases sigmoidally with age and that the yolk mass decreases (Figure 1.3).

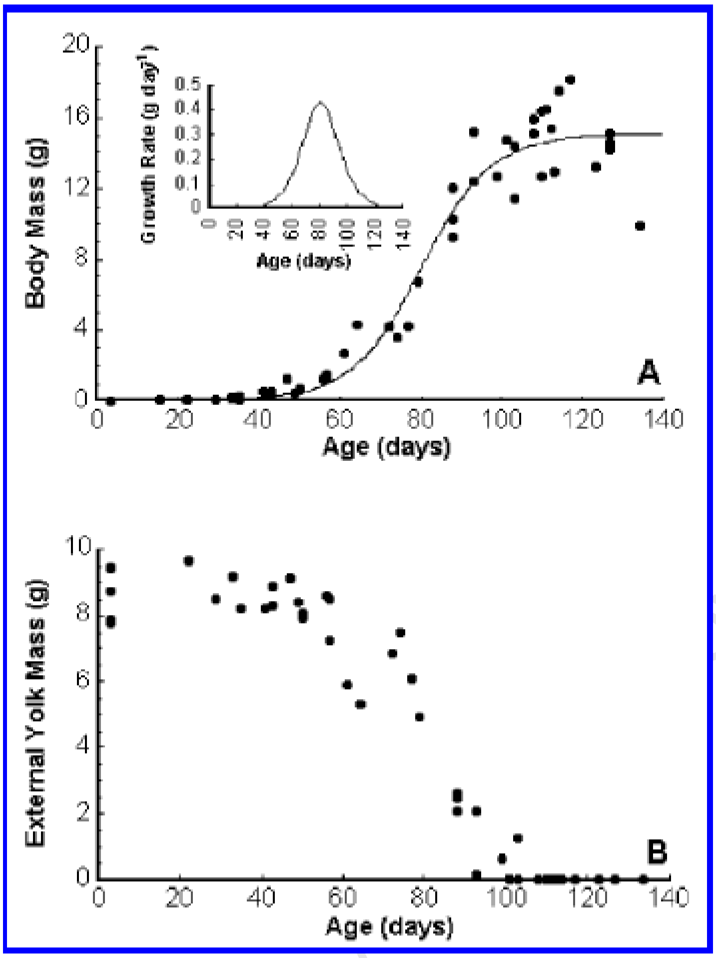

Figure 1.3: Sigmoidal increase in body mass of *Chiloscyllium plagiosum* with age and a decrease in external yolk mass (Tullis and Peterson, 2000. pp 274).

Turbulence and embryo development

Turbulence is described as the movement of molecules through a liquid medium where both potential and kinetic energy are sent from one place to another and then dissipate (Margalef, 1997). This movement provides an opportunity for heat, nutrients, oxygen, waste products and suspended particles to be quickly transported from one place to another and is thus an important feature contributing to the maintenance of life in shallow marine ecosystems (Margalef, 1997). Turbulence is so vital to organisms living in water that Ambuhl (1960) wrote “There is no life without water, and there is no life in water without turbulence in water”. Natural oxygen concentrations in the ocean are mainly regulated by salinity and temperature (SA water quality guidelines, 1996) and are useful aids in determining the biological history of water, such as currents and mixing processes (Drew, 1999). Gaseous exchange occurs at the surface, where air and water meet, and is strengthened through wave action. The concentration and dispersion of oxygen is dependant on its solubility and interaction with other processes of a biological nature (Drew, 1999). Turbulence can thus be defined as an enhancement of gaseous exchange at the surface due to interaction with the atmosphere and increased wave action. The two catshark species that are being compared lay their eggs at different depths. Their differences in egg-laying strategies could possibly affect their rate of development, due to exposures to different oxygen concentration. Not much research to date has been undertaken on the effect of turbulence on the rate of embryo development of elasmobranch fishes and this research aims at identifying whether oxygen is an important water parameter during development.

Fouling and embryo development

Fouling is the result of the settlement of aquatic organisms on a particular surface, either living or non-living. These organisms are known as epibionts. Shark eggs are thus a favourable area for the settlement of epibionts, due to their sedentary nature and continual exposure to seawater. Should fouling occur on the egg cases, it could have implications for the developing embryo by affecting the permeability of the egg case, thus preventing the embryo from receiving the necessary oxygen. Other implications of fouling include the fact that the host may become heavier and less buoyant (Dixon *et al.,* 1981, Cuomo *et al*., 1985, Oswald and Seed, 1986), the elasticity of the egg case can be reduced resulting in an increase in brittleness which allows for easy breakage (Dixon *et al.,* 1981), and fouling organisms can be predated on, thus exposing the host to grazer damage (Dixon, *et al.,* 1981, Oswald *et al.,* 1984, Bronmark, 1985, D' Antonio 1985).

As a result of the potential negative impacts of fouling, many organisms have developed mechanisms for protection against fouling (Wahl, 1987). The types of defences used to combat fouling pressures can be mechanical, physical or chemical. Chemical defenses seem to be the most common form of defense among species that are sedentary and involve the utilization of secondary metabolites (Wahl, 1987). An example of a group of organisms that uses chemical defense against fouling pressures are the echinoderms. Proteolytic enzymes and chondroiton sulphate are found in the cuticle, which deters the adhesion of bacteria. Mucus secretions (which is an example of a mechanical defence) are then thought to remove any bacteria that have managed to adhere to the surface. Examples of physical defenses include shark skin that has microtopographical ridges to prevent the adhesion of microorganisms and marine mammal skin that has a surface tension that is also unsuitable for adhesion (Scardino, 2006). There are some advantages for an organism that is fouled, for example water-retaining fouling organisms protect intertidal organisms from desiccation (Penhale and Smith, 1977) during low tide, while other types of fouling organisms act as camouflage (Stoecker, 1978, Witman and Suchaneck, 1984), as well as chemical camouflage (Fishlyn & Phillips, 1980, Feiarek, 1987). According to Lynch *et al.* (1979), when the host comes into contact with these organisms, it may enhance the host's supply of vitamins and can convert nitrogen to ammonia (Goering & Parker, 1972). This is an important process that allows free nitrogen to be broken down into nutrients that can be readily absorbed by the organism for the utilization in more complex organic compounds. The three known kinds of adaptations against fouling are tolerance, avoidance and defence.

Although some research has been undertaken on the life history of elasmobranchs, little information is available on reproduction and embryo development. A comparative study was therefore undertaken on egg deposition and the rate of embryo development of two endemic nearshore catshark species, *Haploblepharus pictus,* and *Poroderma pantherinum* under different environmental conditions. This would assist in determining whether their reproductive behavior is linked to water parameters (temperature and turbulence). Also, due to the sedentary nature of catshark eggs, they are particularly vulnerable to fouling.

Investigations were made into the fouling pressures of cat shark eggs in the field and its effects on development.

Chapter 2

A comparison of egg laying behavior between *Poroderma pantherinum* and *Haploblepharus pictus*

Introduction

Thirty-three percent of elasmobranchs are oviparous (Compagno, 2005), including skates (Rajiformes) and two shark families —Heterodontidae and Scyliorhinidae, although only the Heterodontids are exclusively oviparous (Powter and Gladstone, 2008). Besides the mode of reproduction, studies of reproductive biology in elasmobranchs can be further subdivided into those investigating sexual maturity, fecundity and reproductive periods (Krebs, 1985). These “rates of demographics” are vital in understanding the life history of an organism. For example, it is interesting to speculate on the relative advantages of ovipary vs vivipary in terms of reproductive success. Oviparous species are able to produce more offspring (Holden, 1973), but since their egg cases are then subject to mortality during development, the ultimate survivorship of hatchlings may not be any higher than occurs in viviparous forms (Shine, 1989; Clutton Brock, 1991). Wourms (1977), who studied the reproduction and development in Elasmobranchs, tried to ascertain whether oviparous species displayed any form of parental care and concluded that the only form of parental care involved was the female selecting suitable breeding sites and the orientation of her egg cases within these sites. Castro (1993) showed that off the East Coast of America *Mustelis Canis* females have preferred areas for egg deposition, known as nursery areas. These are mainly situated in shallow water and have high productivity, ensuring food is easily accessible for juveniles. High availability of food results in rapid growth rates — this would thus be a determining factor when females select suitable breeding habitats (Castro *et al*., 1988).

Dissections of *Heterodontus portusjacksoni* females by McLaughlin and O'Gower (1971) provided evidence suggesting that eggs are laid in pairs, based on the presence of one egg in each oviduct. Other research also suggests that it is common for oviparous elasmobranchs to produce one pair of eggs at the same time (Castro *et al.,* 1988; Ellis and Shackly, 1995; Henningsen, 1999). Oviparity in elasmobranchs can be subdivided into single (external) or multiple (retained) (Nakaya, 1975; Compagno, 1990). Single oviparity refers to a single egg produced in each oviduct, so that the eggs are laid in pairs, and multiple oviparity refers to several eggs retained in the oviduct at one time, so that multiple egg laying occurs. Observations on the reproductive behavior of the Zebra shark *Stegostoma fasciatum* in the Shedd Aquarium from 2004-2008 showed that eggs were laid in 6-8 day intervals, with up to seven eggs being laid at one time (Christopher and Thomas, 2009). This would be an example of a species that exhibits multiple oviparity, which happened over a period of 4-5 months. The female would swim around and wrap the egg tendrils around an erect structure, pulling out additional eggs from the body cavity. Observations on egg laying behavior of *H. portusjacksoni* in the wild showed that females choose breeding sites and deposit their eggs, which are securely lodged, in rocks and crevices. The morphology of the egg case probably plays a role in the egg-laying, as females choose a preferred type of substratum. *H. portusjacksoni* egg cases do not possess tendrils, resulting in oviposition taking place on rocks, where the movement of water assists in securing the egg case in a crevice (Powter and Gladstone, 2008). Other species, such as *H. galeatus,* possess long tendrils to anchor the egg cases to seaweed or sponges (Waite, 1896), thus making a deliberate choice of substratum. Observations on *Scyliorhinus canicula* egg cases have confirmed that the preferred substratum for oviposition is on macroalgae, such as *Fucus spp,* or the base of *Laminaria spp* (Ellis and Shackley, 1997). Erect invertebrates, such as hydroids, sponges and bryozoans, are also used for egg attachment. The toxins these invertebrates produce may also act to reduce fouling pressures and protect the eggs against predators (Dyrynda, 1985) and it is speculated that the egg cases sequester these substances (Dyrynda, 1985). Observations on the depths where species lay their eggs are seldom reported, but *H.*

portusjacksoni lay their eggs between 1 – 20 m, with the majority occurring in the first 5 m (McLaughlin and O'Gower, 1971). There have been similar findings for *S. canicular,* which also lay their eggs at about 5 m water depth (Munchin, 1987) and it is believed that by females inhabiting shallow waters, egg production is stimulated. This therefore suggests, along with other factors, that temperature plays an important part for females when selecting a suitable habitat which is evident in the viviparous grey reef shark *Carcharhinus amblyrhynchos* (Economakis and Lobel, 1998). Observations on embryo development have been undertaken on *S. fasciatum* at the Shedd Aquarium, providing further evidence that temperature influences incubation period and that an increase in temperature results in a decrease in incubation time (Kunze and Simmons, 2004; 2009). Timing of oviposition of a Scyliorhinid, *S. canicula,* has been investigated by Ellis and Shackley, (1997). This could provide a better understanding of not only the reproductive cycle, but the distribution pattern of species, due to shifts in habitat selection as a result of reproduction. By analyzing the amount of fouling on an egg case, the time of oviposition can be determined (McLaughlin and O'Gower, 1971). As a result, along with observations made on the number of adults that frequented shallow reefs and the number of egg cases observed, a definite seasonal pattern for reproduction has been observed by *H. portusjacksoni* (Powter and Gladstone, 2008). Similarly, observations on *S. canicula* adults found more females on shallow reefs in summer and more males in winter (Ellis and Shackley, 1997), providing further evidence of a seasonal breeding cycle among Scyliorhinids. The duration of the breeding cycle according to the number of adults observed could be dependant on water temperatures (Powter, 2006). McLoughlin and O'Gower (1971) reported that *H. portusjacksoni* adults migrate at the end of the breeding season and it is speculated that it is probably to cooler waters. Their return to the same breeding area is due to navigational cues, of which little is known, but rheotactic and olfactory cues, or changes in water temperature, along with knowledge of reefs, could aid in their navigation. Scyliorhinids eggs are produced all year round, but at certain times of the year oviposition is more common (Cross, 1998; Chen *et al.,* 1996; Ellis and Shackley, 1997; Richardson *et al.,* 2000). The ability to produce eggs all year round results from females having the ability to store sperm (Metten, 1939). Habitat selection, depth, temperature and fouling pressures influence embryo development, which could help explain female egg-laying.

Southern Africa is home to a rich diversity of sharks with at least 46 families, 106 genera and 210 species thus far recorded (Ebert *et al.,* 2006). Out of the 160 Scyliorhinid species that exist globally, 16 are endemic to South Africa. However, despite their abundance, very little is known about their biology. Most are oviparous, with the exception of *Galeus polli* and *Halaelurus lutarius,* which exhibit aplacental viviparity and oviparity respectively (Bass *et al*., 1975). *Poroderma* species are distributed from the Western Cape to Southern KwaZulu-Natal (Bass *et al*., 1977), with *P. pantherinum* being more common in the warmer waters of the Eastern Cape and *P. africanum* found mainly in the cooler waters of the Western Cape (Compagno, 1988), although there is a fairly large overlap of these species. *Haploblepharus pictus* is a temperate species distributed from Namibia to False Bay and possibly to the Eastern Cape (Compagno, 1988). There is an overlap with *H. edwardsii,* which is distributed from False Bay to East London. The main focus of this study will be on comparing egg-laying behavior between *P. pantherinum* and *H. pictus,* although some data collected on congeneric species (*P. africanum* and *H. edwardsii*) are included. In oviparous elasmobranchs, it is possible to determine which species an egg belongs to from the morphological characteristics of the egg case. Smith and Griffiths (1997) described the egg cases of *P. pantherinum* as being light brown to greenish brown with thinner tendrils and walls and 7 x 3 cm in dimensions. The egg cases of *P. africanum* are more rectangular, robust and dark brown with dimensions of 9.5 x 4.5 cm. Those of *H. pictus* are purse-shaped with a variation in colour from amber to dark brown. The eggs of *H. edwardsii* and *H. pictus* are very similar, the only difference being that the dimensions in *H. edwardsii* are smaller (38 x 18 mm) and they have distinct pale bands running across the egg case. According to Compagno (2005), all four species are found in the intertidal area down to a depth of 288 m, with the exception of *H. pictus,* which extend to a depth of only 35 m. The incubation period for *P. pantherinum* has been recorded to be 5.5 months (Compagno *et al.,* 2005) and that of *H. pictus* at 3.5 months (Von Bonde, 1945a). Smith and Griffiths (1997) documented hatching success of *Poroderma* and *Haploblepharus* species, based on the proportions of beach cast egg cases that showed evidence of successful hatching. They estimated that *H. pictus* had a high rate of hatching success (61.7%) with low depredation rates (20.8%), as opposed to *P. pantherinum* that had a lower hatching success of 47%, with high depredation rates. These observations may, however, be biased as a result of being based only on those egg cases which became dislodged and cast ashore. Environmental variables could possibly also influence the rate of hatching success.

Little information on habitat selection for oviparous elasmobranchs exists in literature. Observations on habitat selection, the type of substratum on which the eggs are attached, the depths and temperature at which the eggs are laid and the time of oviposition, can contribute to further understanding of reproductive behaviour of sharks in our local waters. By observing egg deposition of various species (more specifically *P. pantherinum* and *H. pictus*), one will be able to determine if different habitats in the ocean are species specific. Female sharks need to ensure the survival of their offspring and habitat selection is therefore very important. Observations on reproductive behavior could contribute significantly to understanding of the life history of elasmobranchs and provide insight into their behavioral ecology. The aim of this Chapter is to document these variables for the species under study.

Materials and Methods

Study area:

The study was conducted around the Cape Peninsula, particularly along its eastern shores, which form the western shore of False Bay. The Bay has a coastline extending for 109 km from Cape Point to Cape Hangklip, a total surface area of 1082 km² and a volume of 44.6 km³ (Spargo, 1991). The deepest part of the Bay is located at the mouth, where it extends down to 80 m and rises steeply to shallower waters on the eastern and western coastlines. The eastern and western coastlines are dominated by rocky shores, whereas the northern coastline is predominantly sandy. The area has a mean Spring tidal range of 1.48 m. Sea temperatures are seasonal, ranging between 16-19°C in Summer and 12.5-14°C in Winter. The warm Agulhas Current influences the water circulation of the Bay, making the eastern side of the Peninsula warmer than the western side. The climate is Mediterranean, with cold wet winters and hot dry summers.

Figure 2.1 shows temperature profiles taken by Atkins (1970) for different seasons in False Bay. It is clear that there is a vertical change in temperature by at least 1°C between 0-10 m, with an even greater temperature change occurring in the summer months. Temperature depth profiles taken by Gründlingh and Largier (1991) suggests that a strong thermocline exists during the summer months.

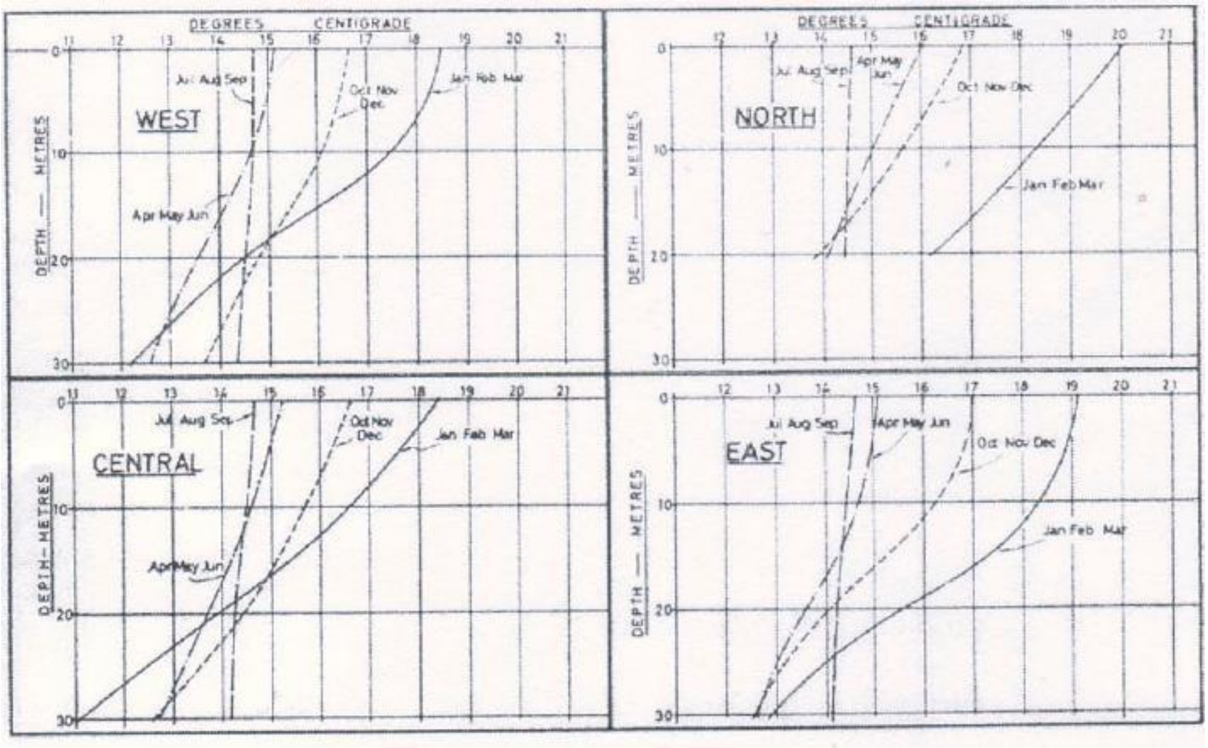

Figure 2.1: Temperature profiles taken at four areas within False Bay, after Atkins (1970).

Experimental methods:

Observations on different habitats where eggs are laid by *Haploblepharus* and *Poroderma* females were undertaken in False Bay, focusing primarily on *H. pictus* and *P. pantherinum*.

An analysis of the depths and temperature at which the eggs were laid, the type of substratum to which the eggs were attached and time of oviposition of each species were undertaken and compared. Data were collected both on SCUBA and on snorkel from January 2011 to January 2012. A total of 68 dives on SCUBA and 14 snorkel dives were undertaken. The snorkeling and dive sites where data were recorded are shown in Figure 2.2. Data sheets were handed out to members of the False Bay Underwater Club, SURG (Southern Underwater Research Group), and other divers who offered to assist in observations on their dives. The following data were recorded: Date; Temperature, Location of Dive; Species; Number of eggs on substratum; Viable or non viable; Type of substratum; Depth; Fouling. The depth was recorded using a pressure gauge, and a dive computer was used to record temperature. While snorkeling, depth was estimated visually in relation to the surface.

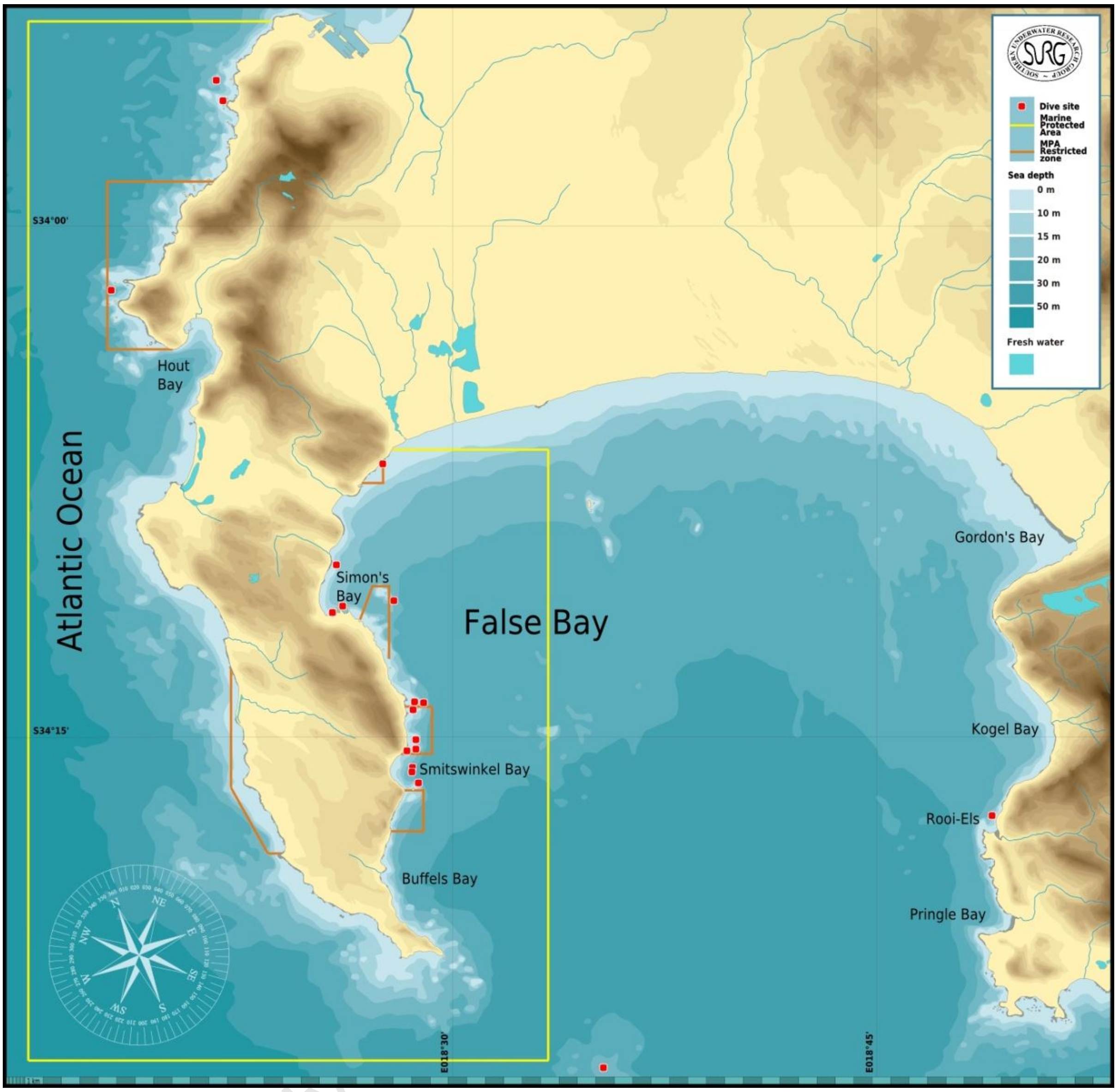

Figure 2.2: Map illustrating the diving and snorkeling sites around the Cape Peninsula. The yellow line indicates the marine protected area and the brown line indicates the restricted zones.

Statistical analysis:

The depth and temperature at which *H. pictus* and *P. pantherinum* lay their eggs were checked for normality by visual inspection (using frequency histograms). The Mann-Whitney U Test was used to test for differences in depth and temperature between the two species, because neither depth nor temperature distributions were close to normal for both species.

Results

The numbers of dives undertaken during each season are given in Table 2.1. Dives included shore entries, boat dives and snorkeling, totaling 76 dives. Conditions were not always favorable, so it was difficult to get the exact same number of dives per season. Most of the dives were undertaken on SCUBA, with the greatest number in winter, followed by autumn and then spring and the lowest number in summer. Summer had the least number of dives due to poor diving conditions i.e. poor visibility and large swell. The most number of snorkel dives were done in autumn, followed by summer. The least number of dives were in spring.

Table 2.1. The number of SCUBA and snorkel dives undertaken per season.

Season	# SCUBA dives	# snorkel dives
Autumn	17	5
Winter	23	3
Spring	10	4
Summer	12	2

Egg laying behavior of *Poroderma spp:*

The total number of eggs observed for *P. pantherinum* was 54 and all the observations were made on SCUBA and none while snorkeling. Due to numerous people collecting data, the duration of the dives was not recorded. This made it difficult to standardize the effort expended per dive. It is assumed that the duration averages out to a similar figure. Out of the 76 dives undertaken, *P. pantherinum* egg cases were observed in 29%. The maximum number of egg cases observed on one dive was 14. This accounted for 26% of the total eggs observed. Statistically, there was no significant difference between the months in which the eggs were observed, due to the large error bars (representing standard deviation) (Figure 2.3). However, the graphical representation shows the highest average number of eggs observed per dive to be between December and February (1.3 ± 3.6) (Figure 2.3). The lowest average was between March-May (0.4 ± 1). The average number of eggs increased gradually (0.9 ± 1.1) between June and August and September and November (1± 1.9).

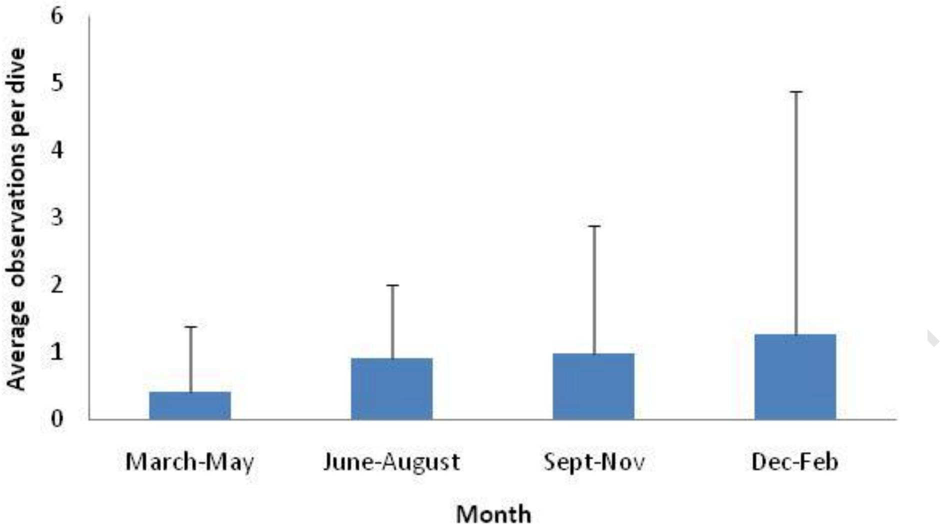

Figure 2.3. Observations of *P. pantherinum* egg cases per dive for each season. The error bars indicate standard deviation.

Eighty percent of the egg cases observed were found between 6 and 20 m depth (Figure 2.4). No observations were made shallower than 5 m. The maximum depth that an egg case was observed was 26.8 m, the minimum 7 m and the average 15.7 m ± 28.82. The depths at which *P. africanum* lay their eggs is also shown in Figure 2.4. The depth range was very similar to that of *P. pantherinum* (6-25 m).

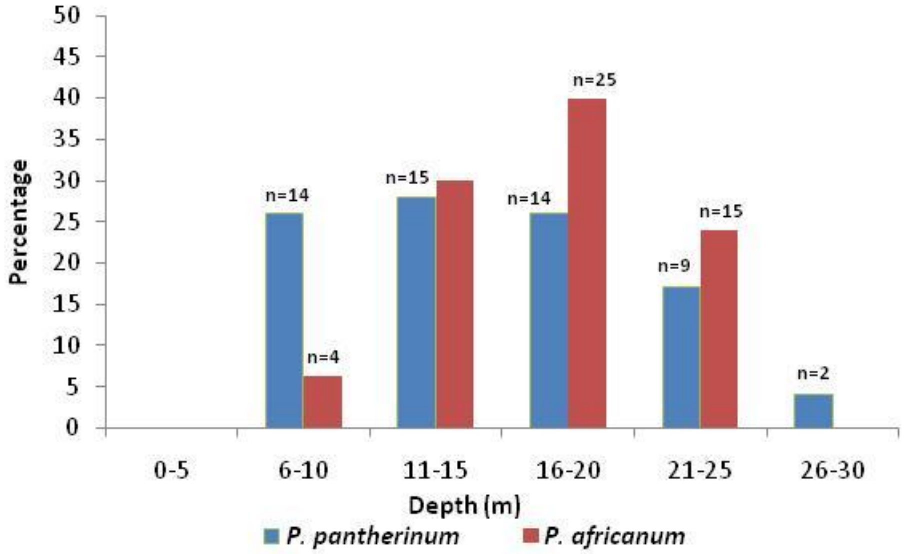

Figure 2.4: Egg laying depths for *P. pantherinum* and *P. africanum*

The preferred substrata on which *Poroderma spp* choose to lay their eggs are shown in Figure 2.5. The majority (89%) of *P. pantherinum* egg cases were found on sea fans (*Leptogorgia palma, Eunicella papillosa*) and similar findings were evident for *P. africanum* (97%). Some (7.4%) *P. pantherinum* egg cases were found lying loose on the reef and on an occasion were found tightly wrapped around kelp. Similarly, 3.2% of *P. africanum* egg cases were found either around kelp or on a hydroid.

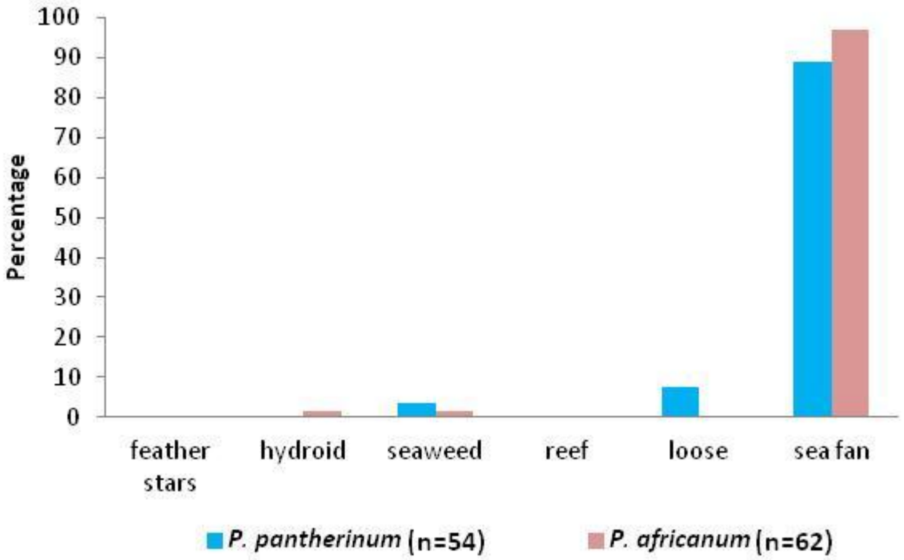

Figure 2.5. The relative abundance of eggs on each substratum on which *Poroderma* eggs were laid.

Ninety-three percent of the egg cases occurred singly, while the remaining 7% were found in pairs (Figure 2.6).

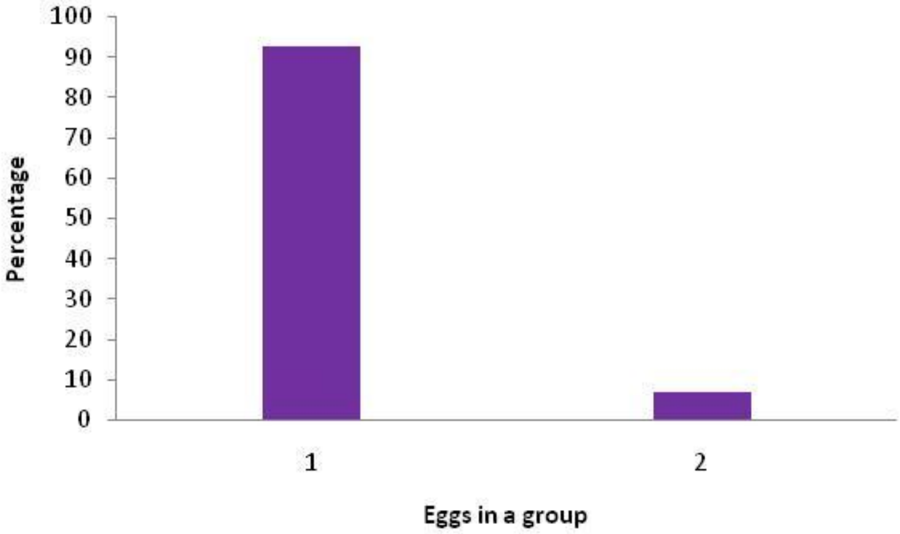

Figure 2.6. Number of eggs found singly or in a group for *P. pantherinum.*

The minimum recorded temperature at which *P. pantherinum* egg cases were observed was 12°C (August) and the maximum 21°C (December) (Figure 2.7). The average temperature where the egg cases were found was 15.7°C. The temperature fluctuated by 3°C throughout the year with a significant increase as summer approached. The temperature where the maximum number of egg cases was found was at both 14°C (n=17) and 19°C (n=17).

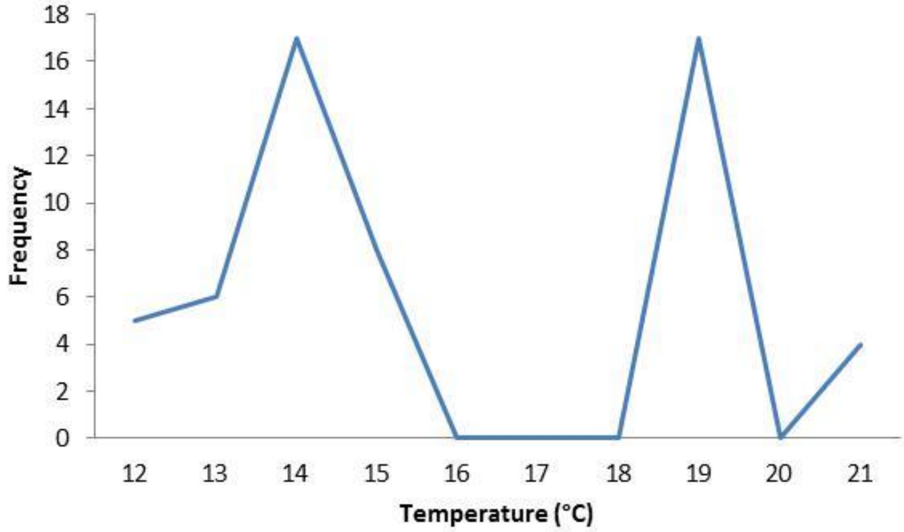

Figure 2.7. Frequency of *P. pantherinum* egg cases observed with temperature.

Egg laying behavior for *Haploblepharus spp:*

Out of the combined snorkels and dives undertaken, *H. pictus* egg cases were observed in 21%. A total of 196 egg cases were observed, with the majority (94%) found while snorkeling. Due to a large swell associated with the South Easterly wind that dominates False Bay (Figure 2.8), opportunities for snorkeling and diving were not always available and could only be done once reasonable conditions presented itself.

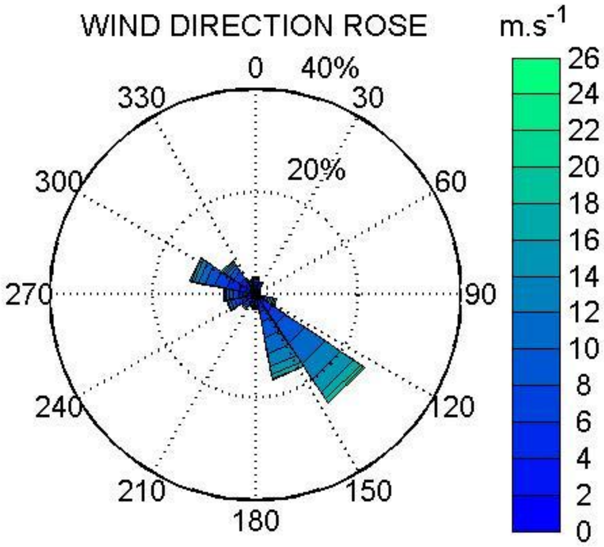

Figure 2.8. A wind direction rose indicating the South Easterly wind dominating False Bay.

The maximum number of egg cases observed on a dive was 40, which accounted for 20% of the total eggs observed. Statistically, there was no significant difference between the months in which the eggs were observed, due to the large error bars (representing standard deviation) (Figure 2.9). However, the graph shows the highest average number of egg cases observed per dive to be between December and February (4.9 ± 11). The observations between March and May were slightly less (3.3 ± 8.5) and the lowest between June and August (1.1 ± 4.7). The observations then started picking up again (1.9 ± 6.4) between September and November.

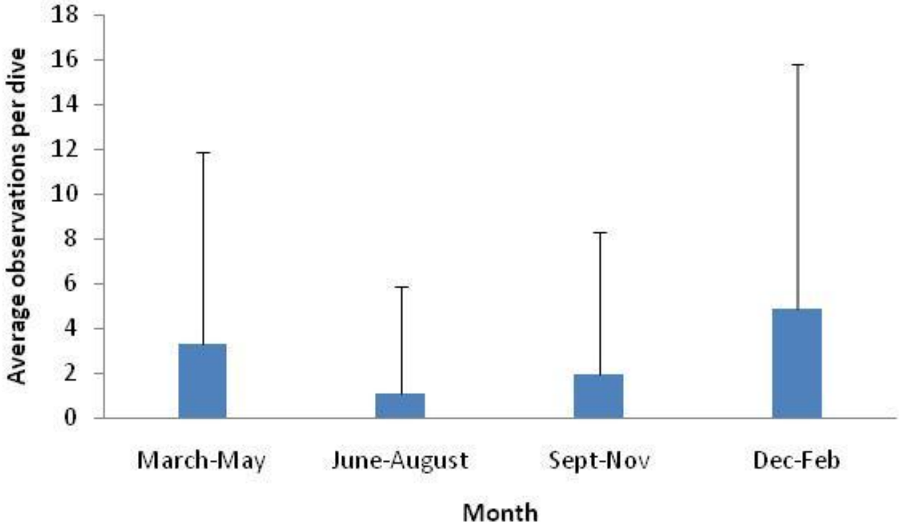

Figure 2.9. Observations of *H. pictus* egg cases per dive for each season. The error bars indicate standard deviation.

The maximum depth at which *H. pictus* eggs were found was 25 m, the minimum 0.5 m and the average 3.94 m ± 15.89. The majority of egg cases (94%) were found between 0-5 m with the remaining 6% found between 11-25 m (Figure 2.10). An indication of the depths at which *H. edwardsii* lay their eggs is also given. Over half the egg cases observed (58%) were between 21-25 m.

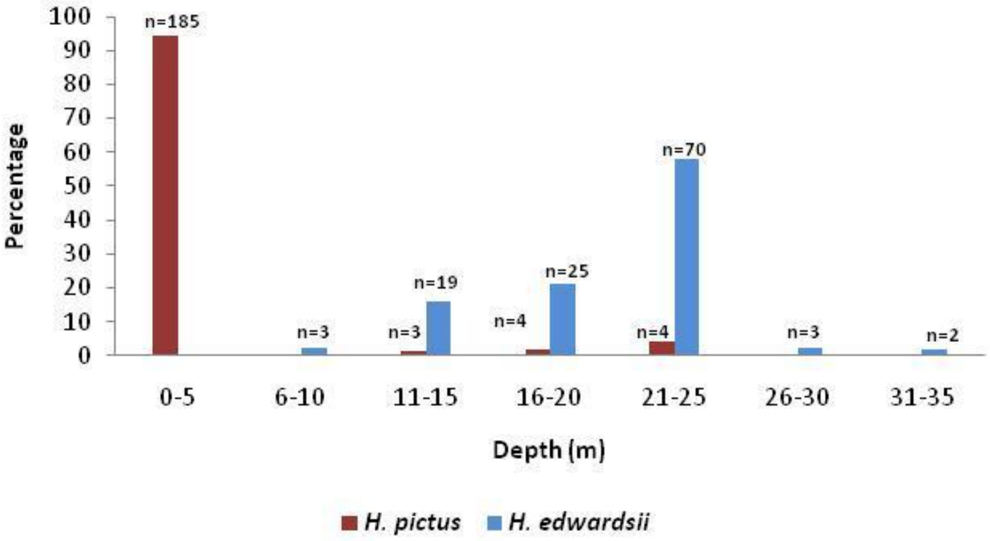

Figure 2.10. Egg-laying depths for *H. pictus* and *H. edwardsii.*

The substrata on which *Haploblepharus spp* choose to lay their eggs are represented in Figure 2.11. *H. pictus* predominantly lay their eggs on one specific type of seaweed *Bifurcariopsis capensis* (97%), followed by seafans (1.5%). On one occasion an egg was observed to be lying loosely on the reef. *H. edwardsii* have a wider range of substrata for egg deposition. Most were observed lying loosely on the ocean floor (57%), or haphazardly attached to the reef (30%). Kelp, hydroids, feather stars and seafans were also selected as substrata for egg deposition.

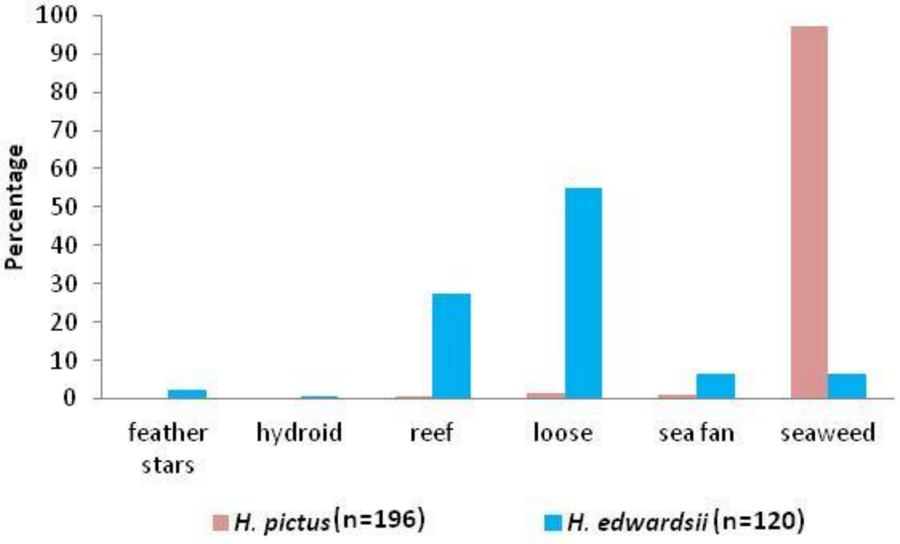

Figure 2.11. Relative abundance of substrata on which *Haploblepharus* eggs were laid.

Numerous egg cases were sometimes observed attached to a single substratum the maximum being eight eggs attached to a single alga (Figure 2.12). Two eggs, however, was the most common number found in a group. It was difficult to determine if all the eggs in a group were laid by the same female, but judging by the amount of growth occurring on the egg cases in the groups, they were very similar in age.

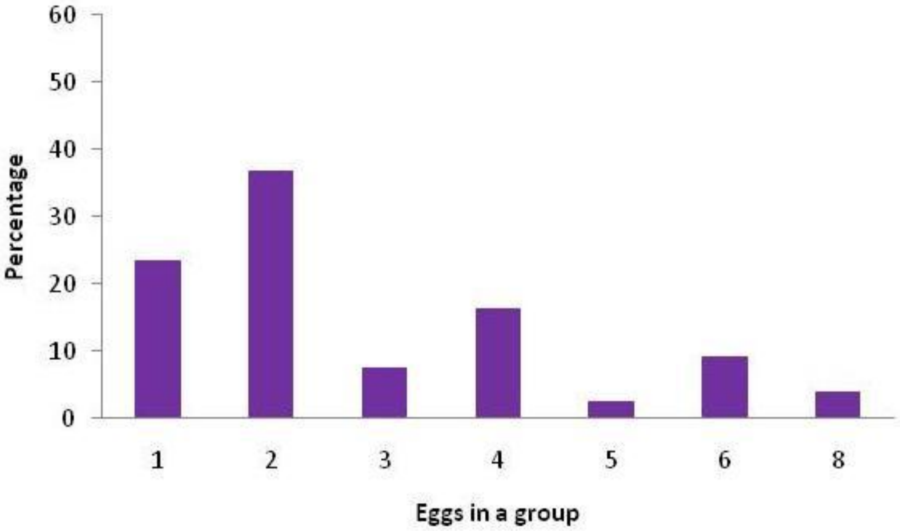

Figure 2.12. Number of eggs observed in a group for *H. pictus.*

The minimum recorded temperature at which the eggs were observed was 12°C (April) and the maximum was 21°C (January) (Figure 2.13). The average temperature at which *H. pictus* females lay their eggs was 15.6°C. The temperature at which the maximum number of egg cases were observed was 18°C (n=56).

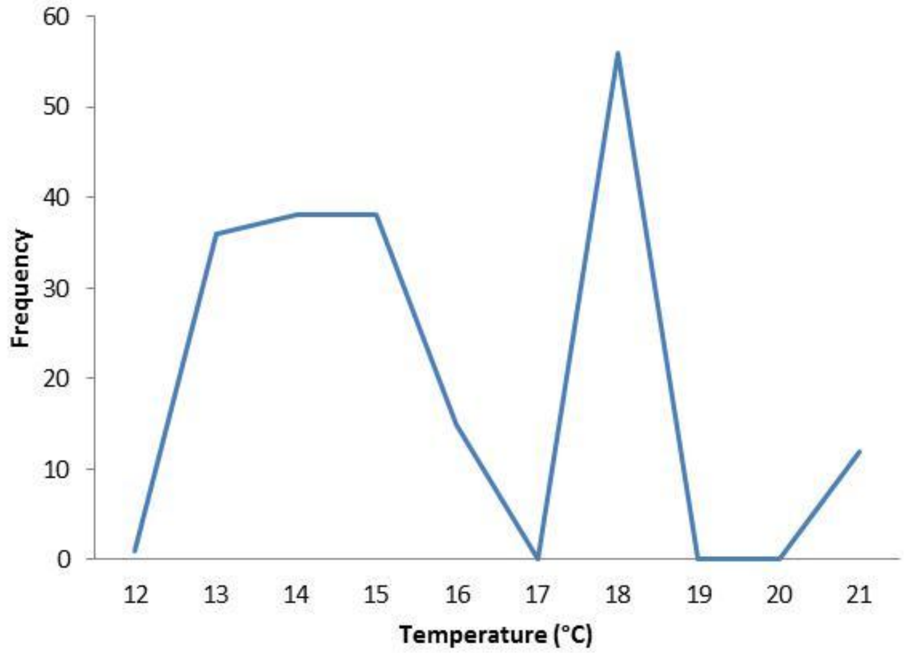

Figure 2.13. Frequency of *H.pictus* egg cases observed with temperature.

Comparison between *H. pictus* and *P. pantherinum*

Normality was checked by visual inspection. Frequency histograms for *H. pictus* and *P. pantherinum* (Figures 2.14 and 2.15) indicate that the data are skewed. There was no significant difference in temperature at which *H. pictus* (Mann Whitney U Test: p=1; u=0) and *P. pantherinum* (Mann Whitney U Test: p=1; u=0) laid their eggs.

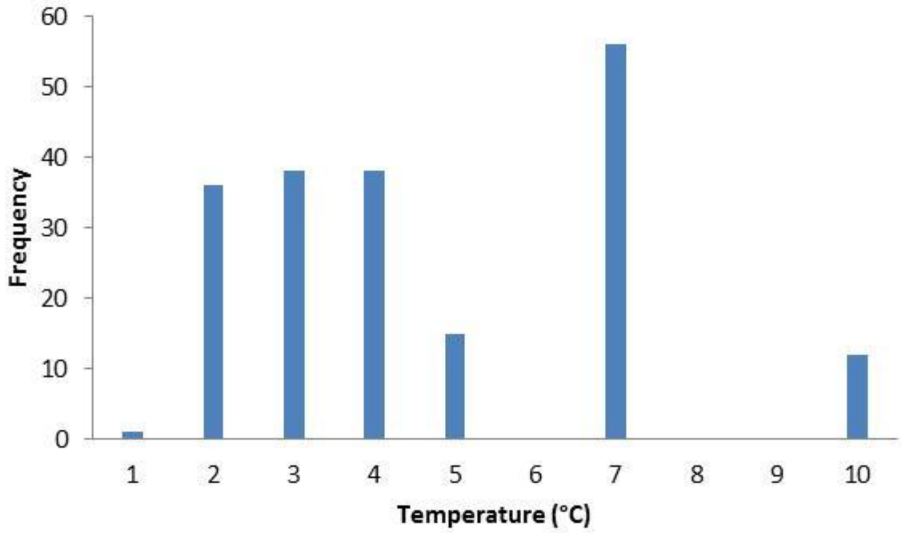

Figure 2.14. Frequency histogram of temperature for *H. pictus*

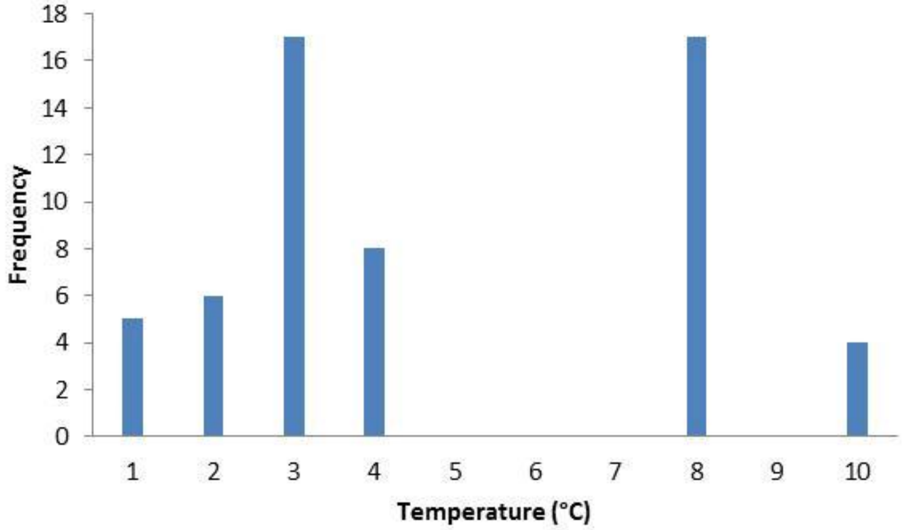

Figure 2.15. Frequency histogram of temperature for *P. pantherinum*

A comparison in egg-laying depths between *Haploblepharus spp* and *Poroderma spp* is highlighted in Table 2. 2. The maximum recorded depths were similar for all species (between 25-35 m), but the minimum recorded depth showed *H. pictus* eggs to be laid in very shallow waters (0.5 m).

Table 2.2. A comparison between the depths at which *Haploblepharus spp* and *P. pantherinum spp* egg cases are found

Species	***H. pictus***	*H. edwardsii*	***P. pantherinum***	*P. africanum*
Maximum	**25**	33.9	**26.8**	25.6
Minimum	**0.5**	8	**7**	7.6
Average	**3.9**	20.5	**15.7**	17.2

Frequency histograms for *H. pictus* and *P. pantherinum* (Figures 2.16 and 2.17) indicate that the data are skewed. There was no significant difference in depth at which *H. pictus* (Mann Whitney U Test: p=1; u=0) and *P. pantherinum* (Mann Whitney U Test: p=1; u=0) laid their eggs.

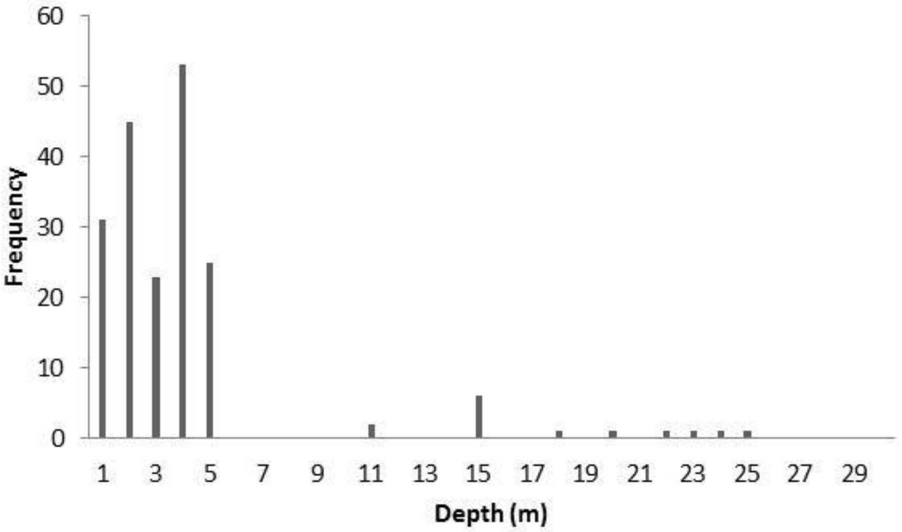

Figure 2.16. Frequency histogram of depth for *H. pictus*

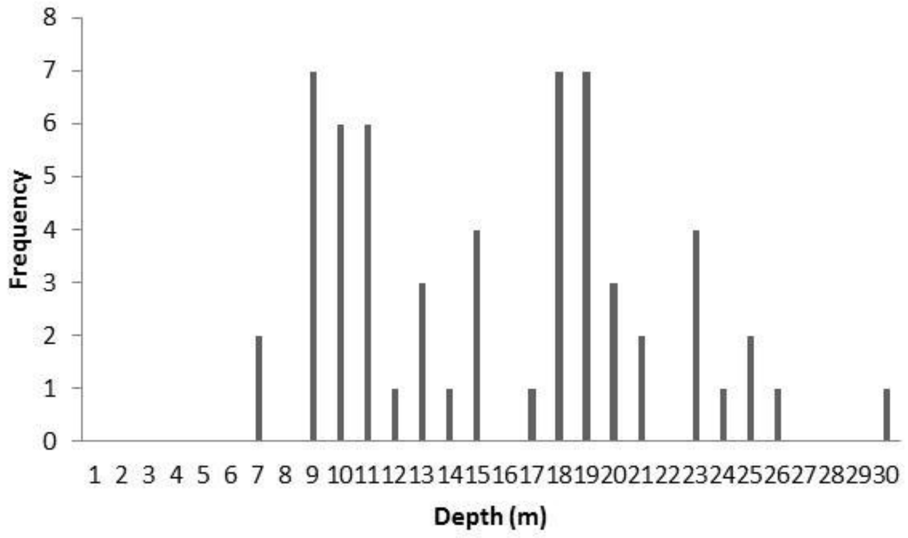

Figure 2.17. Frequency histogram of depth for *P. pantherinum*.

Discussion

i. Season

Sometimes multiple egg cases were observed and more *Haploblepharus* eggs were observed than *Poroderma* eggs. Freshly laid egg cases were observed for both species throughout the year, confirming that Scyliorhinids produce eggs all year round (Cross, 1998; Chen *et al.,* 1996; Ellis and Shackley, 1997; Richardson *et al.,* 2000). Summer did have the highest number of observations per dive (November to February) for *P. pantherinum* and was also the season when the highest numbers of egg cases were observed on a single dive. This is indicative of a nursery area, similar to that which Castro described in 1993. Besides the one dive, not many egg cases were observed in summer compared to winter and spring, where an egg case was observed on almost every dive. This evidence suggests that egg deposition may be more common during the cooler months (June to November). The results for *H. pictus* are slightly different, due to a specific nursery that was identified in Glencairn. The highest number of eggs observed per dive was in the summer months (December to February) although it was the season with the least number of dives. This evidence suggests that egg deposition is more favourable during the warmer months (December – May). A definite seasonal pattern has been observed for *H. portusjacksoni*, but this was determined not only by fouling and the number of eggs, but also by observing the number of adults that frequented the shallow reefs (Powter and Gladstone, 2008). Further investigations should be undertaken to determine a definite breeding cycle for *H. pictus* and *P. pantherinum* by analyzing growth cover on the egg cases and recording the number of females inhabiting the area.

ii. Depth

H. portusjacksoni and *S. canicular* lay most of their eggs in the first 5 m of water (McLaughlin and Gower, 1971; Munchin, 1987). This is very similar to the depths at which *H. pictus* eggs were found (average depth 3.7 m). *H. portusjacksoni* are also known to lay their eggs deeper than 5 m, reaching similar depths to that of *P. pantherinum* (average depth 15.7 m).

iii. Substratum

Both *H. pictus* and *P. pantherium* females selectively attach their eggs to specific substrata. For *H. pictus* it was the seaweed *Bifurcariopsis capensis* and for *P. pantherium* it was seafans. Their choice of substrata could assist in concealing the eggs from predators and possibly deter fouling (Dyrynda, 1985). *Halaelurus galeatus* eggs possess long tendrils similar to those of *H. pictus* and *P. pantherinum* and have been observed to have a preferred choice of substratum, mainly seaweed or sponges (Waite, 1896). Erect invertebrates, such as hydroids and bryozoans, have also been observed as places for female *S. canicular* to attach their eggs.

iv. Number of eggs in a group

Dissections undertaken on oviparous elasmobranches suggest that eggs are laid in pairs as a result of the presence of an egg in each oviduct (McLaughlin and Gower, 1971). It was difficult to determine if this was the case for *P. pantherinum,* because the average number of eggs found on the substratum was one. The females may, however, have attached one of their eggs to a chosen sea fan and then moved over to another sea fan to lay the next egg. It is possible that *H. pictus* and *P. pantherinum* females exhibited different modes of oviparity. For *P. pantherinum* it could have been single (where eggs are laid in pairs) due to the presence of only one egg on the substratum, and for *H. pictus* it could have been multiple (where several eggs are retained in the oviduct at one time, so that multiple egg laying occurs) due to numerous eggs found on one substratum. On one occasion there were as many as eight freshly-laid *H. pictus* eggs. However, there is insufficient evidence to suggest that the eggs were laid by the same female, as no females were observed actually laying eggs. This would therefore need to be further investigated and it is suggested that genetic analyses could be undertaken to test whether eggs on a group are laid by the same female. The Zebra shark *Stegostoma fasciatum* lay up to seven eggs at one time (Christopher and Thomas, 2009). *Poroderma pantherinum* and *H. pictus* females may have similar egg-laying behavior to *S. fasciatum* where the female swims around and wraps the egg tendrils around an erect structure (Christopher and Thomas, 2009).

v. Temperature

The temperature ranges at which both *P. pantherinum* and *H. pictus* egg cases were observed were similar (12-21°C). There were, however, greater fluctuations in temperature at which *P. pantherinum* egg cases were observed. A possible explanation is the differences in depth at which the egg cases were observed (average depth for *P. pantherinum* was 15.7 m ± 28.8 and for *H. pictus* was 3.7 m ± 15.9). Evidence of a change in temperature with depth was given by Atkins (1970). Increased temperature stimulates egg production and decreases the incubation period (Chapter 3). Temperature, therefore, plays an important role for females when selecting a suitable habitat (Econonakis and Lobel, 1998) and it has also been suggested that the duration of a breeding cycle could be dependant on water temperatures (Powter, 2006).

These differences in egg laying behavior between *H. pictus* and *P. pantherinum* would result in the embryos being exposed to a different set of environmental conditions. Further investigations on how variables such as temperature and turbulence influence growth and development can provide further clues as to why different species lay their eggs where they do.

Chapter 3

Effect of temperature and turbulence on the growth rate of *Poroderma pantherinum* and *Haploblepharus pictus* embyros

Introduction

Elasmobranchs exhibit all modes of reproduction found in vertebrates with oviparity considered to be the least advanced mode (Carrier *et al*., 2004). This mode of reproduction results in the embryo being deposited outside the female's body in a protective capsule comprised of keratin-like collagen (Brown, 1975). The nutritional requirements are obtained from a yolk sac within the egg capsule and inorganic substances and water are obtained from the surrounding water (Amarosa, 1960). There are four families of sharks that reproduce through oviparity—Heterodontidae, Orectolobidae and Scyliorhinidae (Wourms, 1977). Egg cases of Scyliorhinidae and Proscylliidae catsharks are depressed, sub-rectangular and elongated, with rounded undulated sides, a truncated concave or convex anterior or vestibular end (through which the hatching shark escapes), a narrow posterior terminal end, usually a pair of short to long horns with tendrils at both ends, and generally a constriction on the capsule near the vestibular end (Compagno, 1988). Scyliorhinidae eggs have been described by Ebert *et al*. (2006) as having a pair of respiratory fissures at both the anterior and posterior ends of the egg case. The fissures are closed by albumen during the first few months of embryo development. In order to better understand embryonic development of elasmobranchs, studies on oviparous species are of particular interest, due to the ability to monitor their development without the use of lethal methods. Scientists can study the different stages of development, including morphological changes, length vs weight relationships, and energetic, respiration and ventilation processes, throughout incubation (Rodda, 2000). Stages have been assigned to embryos during development, which act as a useful guide when describing the progress of development of the embryo. However, insufficient data have been recorded on most of these parameters (Clarke, 1926; Baranes and Wendley, 1981; Gilmore *et al*., 1983; Natanson and Calliet, 1986; Yano, 1992).

Although stages of embryonic development have been recorded in certain viviparous species caught opportunistically during fishing operations, such as for the Sandtiger shark *Odontaspis taurus* (Gilmore *et al.,* 1983) and the Pacific Angel shark *Squatina californica (*Natanson and Calliet, 1986), not much information has been documented on developing embryos held under controlled constant temperature conditions. First observations on developing shark embryos under controlled conditions were made by Smith (1942) who documented the development of the Japanese shark *Heterondontus japonicas*. Ballard (1993) made observations on *Scyliorhinus* embryos at a temperature of 16°C and was able to supply qualitative data on embryo development. He also attributed stages, but focused mainly on the prehatching phase of development (first three months). He described a total of 34 stages for the embryo development of *S. canicula* with only a few embryo measurements.

Harris (1952) studied the rate of development of *Scyliorhinus canicula* and made a comparative study based on observations at two different temperatures. He determined that the rate of development was 1.64 times longer at a cooler temperature of 11.5°C as opposed to a warmer temperature of 15.5°C. From this information he was able to deduce that there were difficulties in determining the incubation period for oviparous species in nature, due to changes in temperature and exact date of oviposition.

Rodda and Seymour (2008) also confirmed the effects of temperature on incubation period by using *Heterodontus* eggs. A total of 15 stages were assigned to the embryo during development under different temperatures (18, 20 and 22°C). The results indicated that an additional 90 days was needed for the embryo to reach full development at a temperature of 18°C as opposed to 22°C, when development took 291 days. Temperature seemed to have no effect on the opening of the mucous plugs. The time it takes for the albumen to dissolve, however, seems to vary amongst species, with *Scyliorhinus canicula* taking *c*70-80 days to open (Ballard *et al.,* 1993), and *Heterodontusportus jacksoni c*120 days (Rodda and Seymour, 2008). The fissures are then completely open to allow the entry of oxygenated water and removal of metabolic wastes. Some water also enters the egg case through osmotic diffusion, but not sufficient to assist in waste exchange (Hornsey, 1978; Koob and Cox, 1993, Thomason *et al.,* 1996).

This study aims at documenting the morphological changes in development of *Haploblepharus pictus* and *Poroderma pantherinum* embryos and how temperature and turbulence influence the growth rate. It was decided to use these two species due to their abundance in the Western Cape. *Poroderma* species are distributed from the Western Cape to Southern KwaZulu-Natal (Bass *et al.,* 1977a) with *P. pantherinum* being more common in the warmer waters of the Eastern Cape, where it inhabits areas on the continental shelf to upper slope and in the intertidal area down to 282 m. Compagno *et al.* (2005) reported that an egg case reared in captivity had an incubation period of 5.5 months. *Poroderma africanum* are found mainly in the cooler waters of the Western Cape, however, there is a fairly large overlap of these species (Compagno, 1988). *Haploblepharus* are temperate species distributed from Namibia to False Bay and possibly to the Eastern Cape (Compagno, 1988). *H. edwardsii* and *H. pictus* are the two common species found in the Western Cape. *H. pictus* are found in the intertidal down to 35 m in kelp, rocky and sandy habitats (Compagno *et al.,* 2005). Their diet consists of bony fishes, sea snails, cephalopods, crustaceans, polychaetes, echinoderms and algae. Egg development in captivity was observed to be 3.5 months (Von Bonde, 1945a). Research has been undertaken on *Haploblepharus* species in South Africa by Von Bonde (1945a) who described the female and male reproductive organs and presented some qualitative data on embryo development. There was taxonomic confusion at this time as he referred to the species in his study as *H. edwardsii*, but his photographs clearly indicate that he was in fact dealing with *H. pictus*. He discovered that after just 10 days the embryo was attached to a prominent umbilical cord and was 2 mm long. He described the vitelline circulation as being very similar to that of a chick embryo and observed the yolk to progressively decrease in size, while the embryo increased in size. He also observed the appearance of the external gill filaments early in development and that these disappeared 50 days into development. Evidence suggests that the gill filaments serve a dual purpose in that they also play a role in urea retention until the embryo is capable of osmoregulation (Smith, 1936; Read, 1968, Kormanick, 1993).

This is the first time that a properly replicated study will be undertaken to compare the development times for *P. pantherinum* and *H. pictus*. The effects of temperature and turbulence on embryo growth and survivorship could help explain why these two species lay their eggs where they do. A description of the development stages will also be given, which has not been done on these species before. A comparative study on embryo development under controlled conditions can ascertain whether species have different growth rates when embryos are exposed to the same controlled conditions.

Materials and Methods

Egg development was monitored both in the laboratory, under various sets of constant controlled conditions, and under naturally-varying conditions in the field, in order to compare the rate of development under each set of conditions and between two species.

i. Collection of experimental material

Adult *Poroderma pantherinum* were kept in captivity at the Department of Agriculture Forestry and Fisheries Research Aquarium in Sea Point, Cape Town. An average of 30 eggs a month were collected for a year and a half (from February 2010 to May 2011) and transported in a bucket to the closed aquarium system at the University of Cape Town. *Haploblepharus pictus* egg cases were collected in the field on SCUBA and while snorkeling in Glencairn and Woolley’s Pool in False Bay. Freshly laid eggs were mostly taken and if this was not possible then the embryos were measured using the laboratory technique explained below and an age was determined according to previous embryos developing embryos in the laboratory. Optimal diving conditions were not always prevalent, so eggs were collected whenever possible. Both *P. pantherinum* and *H. pictus* eggs were used in laboratory and field experiments.

ii. Laboratory experiment

The laboratory experiments were undertaken in the aquarium at the Zoology Department, University of Cape Town, which is a closed system fed by a 3000 l header tank. Seawater is fed by gravity through a pipe running midway along the wall and fitted with several valves which, when opened, allows the seawater to run into experimental tanks on a bench. The water then runs off the bench into a catchment and filter chamber below the bench, from where it is pumped back to the header tank. The water is changed bimonthly using fresh seawater from the aquarium at the Department of Agriculture, Fisheries and Forestry in Sea Point, and the temperature is kept constant at 14°C.

The setup for the experiment consisted of a smaller header tank (150 l plastic container) placed on the bench on top of a concrete block under each of the valves. Three holes were drilled on one side of the tank and an elbow fitting fastened into each hole. A 50 l plastic container was then placed under each fitting. Water was able to flow from the header tank through the fittings and into each 50 l tank. There were three replicates made, giving nine 50 l tanks in total. Each tank was divided into two separate areas using a plastic barrier into which holes were drilled. The one side of the tank, the "turbulent side" contained an air-stone and a submersible pump, and the other side, the "still side" lacked circulation or aeration. The holes in the barrier allowed for a small amount of water circulation to pass through the tank to the still side. Three of the tanks contained heaters in order to raise the temperature to 17°C. Two controlled conditions (14°C and 17°C) were now available to monitor any differences in embryo development.

Eggs were attached to plastic mesh cylinders placed in both the turbulent and still sides of each 50 l tank. A total of 18 mesh cylinders were used. Waterproof paper was used to label each egg. A hole was punched into the paper and a curtain hook inserted through the hole. The tendrils on the top of the egg case were then securely fastened around the curtain hook and the egg pegged to the mesh cylinder. A total of five eggs were pegged around each plastic mesh, resulting in 10 eggs occupying each tank.

To collect embryo total length data, the eggs were placed individually in a transparent container and the embryo illuminated by a torch from below. A ruler was placed alongside the egg as a scale. Due to the curved nature of the embryo body, still pictures were needed in order to obtain accurate length measurements. A camera was set up on a tripod and photographs of the developing embryos taken on a bi-monthly basis. The photographs displayed the date at which they were taken. Once all the eggs were photographed, the photographs were downloaded onto a computer. A piece of string was placed over the curved body of the embryo on the photograph to establish the total length. The string was then measured against the image of the ruler which had been placed next to the egg at the time of photographing. The measurements were recorded on the data sheet and then entered into an excel spreadsheet.

Eggs of both species were sacrificed at different stages of development to obtain the wet weight of embryo, length of embryo, width of head, weight of yolk and diameter of yolk. Live eggs were killed by placing them in a freezer overnight before dissection.

Once the neonates had hatched, their weights and lengths were recorded.

iii. Field experiment

Eggs were deployed in the field so that a comparative study could be made of embryos developing under constant controlled conditions and embryos developing under naturally-varying conditions in the wild. Field observations and collecting stations for *H. pictus* were at two locations around the eastern side of False Bay. Woolley's Pool, an intertidal area near Kalk Bay, was used for field observations and as a collecting station for the eggs. Twelve live *H. pictus* eggs were fastened to the same substratum on which they naturally lay their eggs with the aid of cable ties. Two temperature loggers (Tidbit v2) were also deployed near the eggs to record the temperature. Another intertidal collecting station was Glencairn, 5 km south of Woolley's Pool. Both areas have steep intertidal profiles with narrow surf zones. The substratum is comprised mostly of sandstone and the rocky shorelines are exposed to continual wave action. A narrow kelp reef runs adjacent to the shoreline. The maximum depth in this area was 6 m and it was observed to be a nursery for *H. pictus*. Twelve live *Haploblepharus* eggs were deployed in this area. Their tendrils were spliced through a 1 m rope, which was then securely fastened with cable ties. The one side of each rope was attached to a heavy metal link and the other to a buoy. This allowed the eggs to hang vertically in the water column. The link was then submerged at a depth of 3 m at Glencairn. A temperature logger was securely fastened to the link with a metal shackle.

Field observations for *P. pantherinum* eggs were also undertaken at two locations. One area was at a well-known dive spot called Castle Rocks, located just south of Simons Town towards Cape Point. It was chosen because it was easily accessible from the shore and it reached a suitable depth of at least 10 m. Fifteen live *P. pantherinum* eggs were attached to kelp with the aid of cable ties, along with a temperature logger (Tidbit v2).

The second locality for field observations was in the yacht basin at Simons Town Harbour. This area was chosen as an observation site for *Poroderma* eggs mainly because the eggs could be securely fastened to the moorings in the jetty without the fear of them being washed away. The chances of anyone tampering with the experiment were also less likely, due to controlled access into the yacht basin. This area also attained a depth that was needed to deploy *P. pantherinum* eggs (10 m), as this is the average minimum depth at which these species lay their eggs (Chapter 2). A total of 18 live eggs were deployed with their tendrils securely spliced through a 1 m rope. A buoy assisted in keeping the rope vertical in the water column. Two temperature loggers (Tidbit v2) were securely fastened to the moorings to record temperature.

The eggs were harvested at three different ages during their development - at three months, five months and seven months. Any live embryos were removed from the egg case, frozen overnight and dissected to record morphometric data in the same way as for the eggs sacrificed in the laboratory.

iv. Statistical analysis

Differences in growth rate among the four controlled environments for the two species were examined by plotting 95% confidence intervals. Bartlett's test was used to check for homogeneity of variances among the four environments (Zar, 2000). When parametric test assumptions were not met, two-way ANOVA was used to test for differences in growth rate among treatments (Zar, 2000). Where p assumptions were not met, Kruskal Wallace was used.

Multifactorial ANOVA was used to compare significant difference in weight and time of hatching of juveniles that were exposed to different controlled conditions.

Results

Rate of development for P. pantherinum

A total of 420 *P. pantherinum* eggs were used for this experiment, however, due to a high mortality rate, data were only used for egg cases where three or more sequential measurements could be taken. Representations of the length-age relationships for *P. pantherinum* held under four controlled environmental conditions are given in Figures 3.1-3.4. The four conditions included two temperatures (14°C and 17°C) each under both turbulent and still conditions. The large orange blocks indicate the size and age of the embryos that reached full development and were ready for hatching. The results indicate that length-age relationships were linear in all cases. The growth rate was calculated using a linear growth function, which also assisted in calculating mean growth rate and variance. The mean growth rate in 14°C still and turbulent conditions was 0.05 ± 0.0002 cm/day (n=18) and 0.05 ± 0.0001 cm/day (n=19) respectively. In 17°C turbulent and still conditions, the average growth rate was 0.07 ± 0.0005 cm/day (n=9) and 0.06 ± 0.0002 cm/day (n=7) respectively. The Bartlett's Test for homogeneity showed that the variances were homogeneous ($B_c = 2.195$).

There was a significant difference in growth rate with temperature (F=9.59; p=0.003). However, there was no significant difference between turbulent and still conditions (F=0.05; p=0.81) and the interaction among the four environments (F=0.33; p=0.56). At 14°C it took the embryos an average of 150 days to reach a length of 7.5 cm, whereas at 17°C (turbulent) it took an average of 107 days to reach the same length (Figure 3.4). Thus development of *P. pantherinum* embryos was 1.4 times faster in a temperature raised by only 3°C.

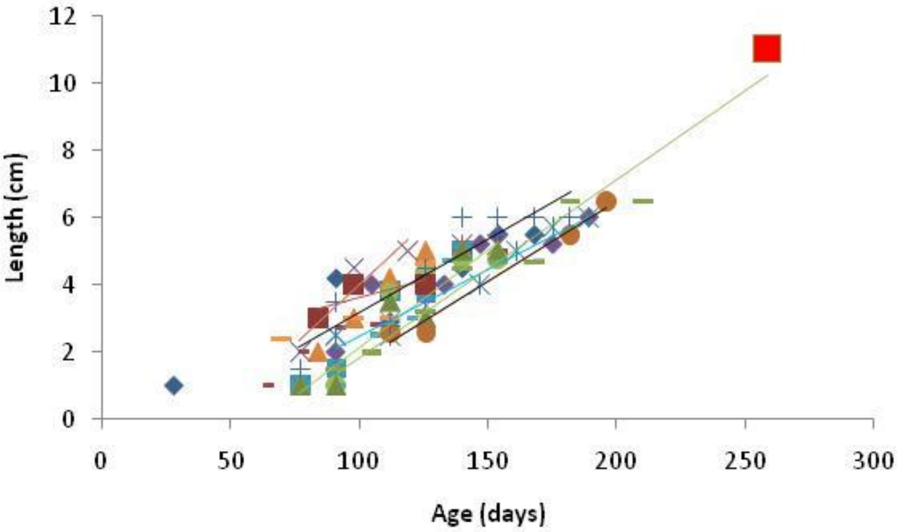

Figure 3.1: Length-age relationship for *P. pantherinum* in 14°C still conditions

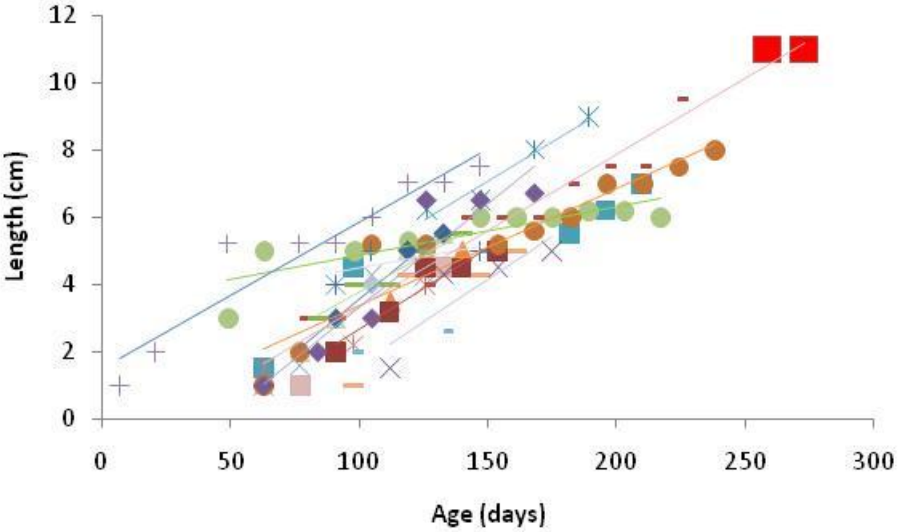

Figure 3.2: Length-age relationship for *P. pantherinum* in 14°C turbulent conditions

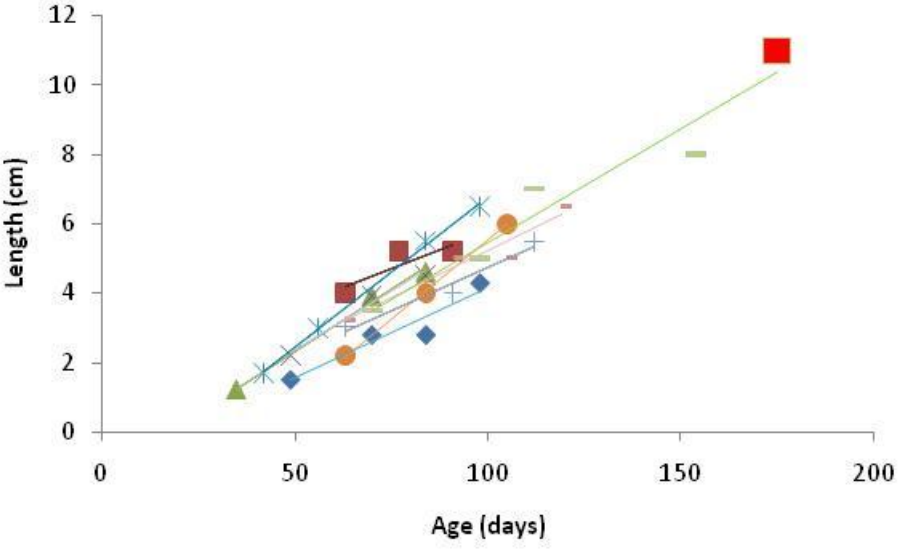

Figure 3.4: Length-age relationship for *P. pantherinum* in 17°C still conditions.

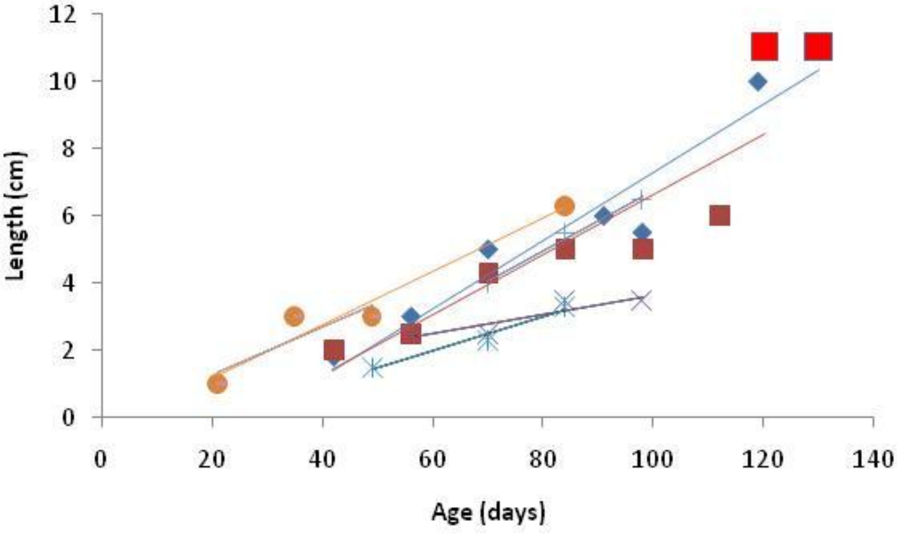

Figure 3.3: Length-age relationship for *P. pantherinum* in 17°C turbulent conditions.

Rate of development for H. pictus

A total of 80 *H. pictus* eggs were collected in the field and used for this experiment. Figures 3.5 - 3.8 represent the length-age relationship for *H. pictus* under both turbulent and still conditions where the temperature was held at 14°C or 17°C. The relationships were all linear. Bartlett's Test for homogeneity showed that the variances were not homogenous ($B_c = 261.02$). At a temperature of 14°C, still conditions produced a mean growth rate of 0.05 ± 0.0001 cm/day (n=9) and turbulent conditions produced a mean growth rate of 0.04 ± 0.0003 cm/day (n=14). At 17°C the mean growth rate was 0.09 ± 0.001 cm/day (n=10) in turbulent water and 0.07 ± 0.001 cm/day (n=6) in still water. There was a significant difference in growth rate between the two temperatures (p=0.000) (Table 3.1). There was, however, no significant difference in growth rate between turbulent and still conditions (p=0.806). The rate at which H. *pictus* embryos developed was 1.8 times faster in a temperature raised by only 3°C.

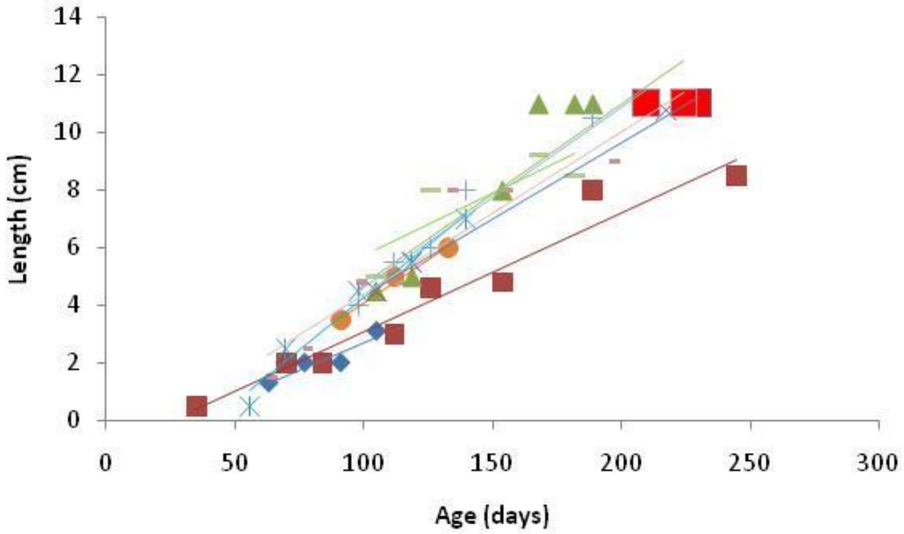

Figure 3.5: Length-age relationship for *H. pictus* in 14°C still conditions

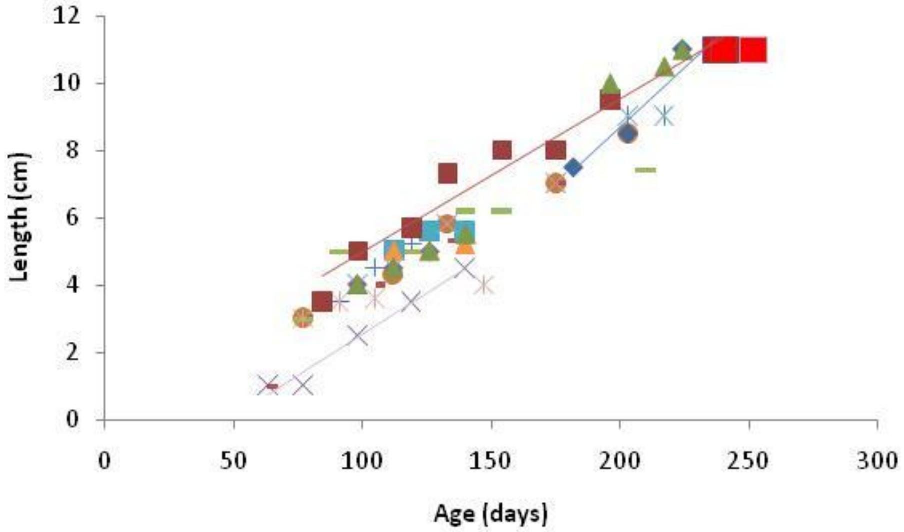

Figure 3.6: Length-age relationship for *H. pictus* in 14°C turbulent conditions

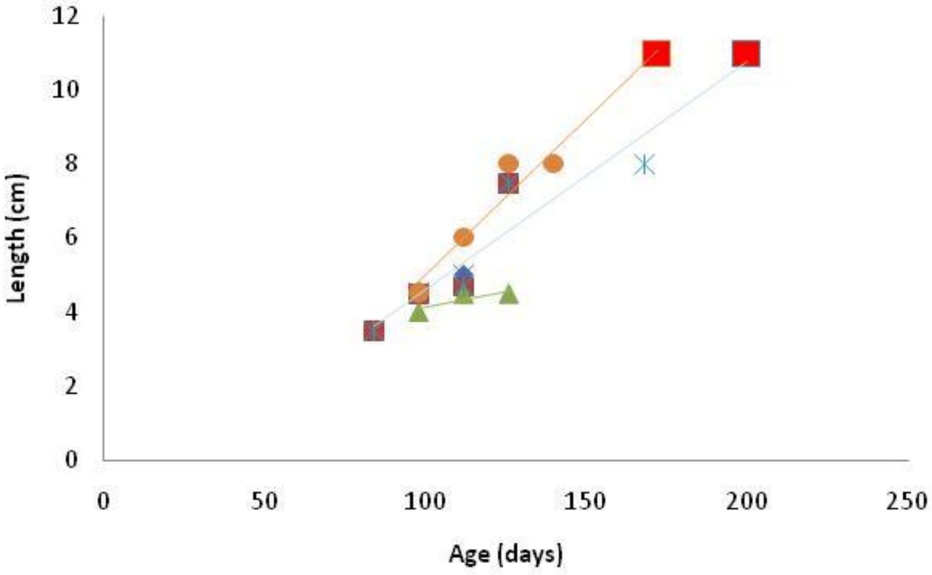

Figure 3.7: Length-age relationship for *H. pictus* in 17°C still conditions

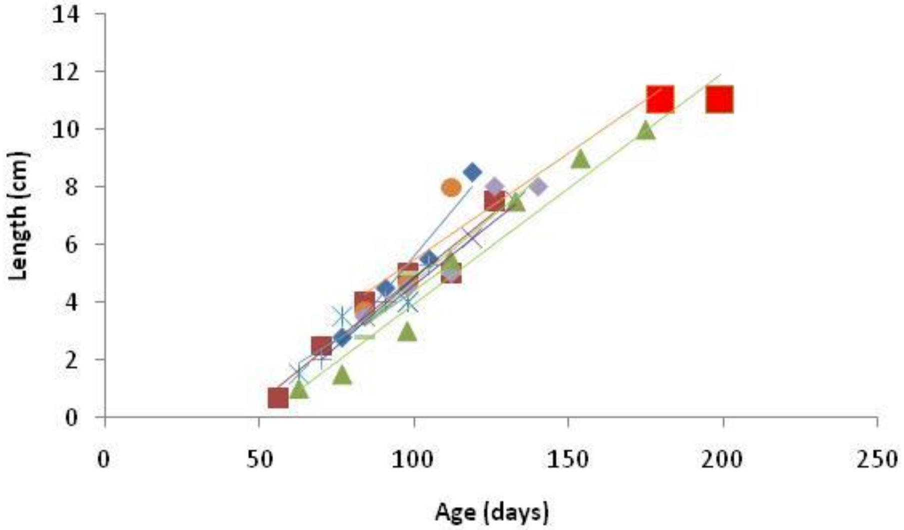

Figure 3.8: Length-age relationship for *H. pictus* in 17°C turbulent conditions.

Table 3.1. H and p values for *H. pictus* growth rates using Kruskal Wallace

Condition	**H**	**P**
Turbulent and Still	0.06	0.806
14°C and 17°C	19.475	0.000

When comparing the performance of both species under controlled conditions, there was no significant difference (F=2.55; p=0.114).

Hatching success

The mortality rate for both species was analyzed using a regression analysis. For *Poroderma sp*, the results indicate that the mortality was higher at 17°C with a rate of 0.21 ± 0.164/day and 0.20 ± 0.372/day for turbulent and still conditions respectively, and 0.11 ± 0.473/day and 0.07 ± 0.095/day for still and turbulent conditions at 14°C. A possible explanation for the high mortality rate at 17°C was because the aquarium pump broke down twice, preventing water circulation through the tanks. This caused the water temperature to rise briefly to as much as 24°C, killing many of the eggs. Another reason could be due to increased nitrate levels as a result of crabs *Carcinus maenas* that were sharing the same water circulation system being overfed. The results for *Haploblepharus sp* also indicate that mortality was higher at 17°C with a rate of 0.15 ± 0.264/day for turbulent and 0.15 ± 0.493/day for still conditions. The rate is slightly lower than for that of *Poroderma sp,* indicating that *Haploblepharus sp* can withstand higher temperatures. The mortality rate at 14°C was much lower than for *P. pantherinum* at 0.01 ± 0.126 cm/day in turbulent and 0.05 ± 0.211/day in still conditions.

Morphological changes during development for P. pantherinum and H. pictus

Developing embryos of each species were sacrificed during each month of development in order to describe the morphological changes occurring. The embryos were all taken from the 14 °C turbulent tanks in order to get an accurate analysis of the growth rate at development under one set of conditions.

1) Morphological characteristics of *P. pantherinum* during development (Figure 3.9).

At 2 months

a. External gill filaments present, eyes starting to develop, body is slender and opaque.

At 3 months (both hatching slits of egg case now open; n=8)

b. Gill slits visible, mouth open to the outside, pelvic and anal fins present, body opaque, increased width, dark pink external gill filaments visible, embryo movement short and pulsating.

4-5 months

c. Internal organs starting to develop, mouth developed, bright red external gill filaments still present (0.7 cm).

At 6 months

d. Microscopic white external gill filaments visible but almost completely absorbed, eyes well developed, pigmentation visible as black bands traversing body.

At 7 months

e. Eyes and gills fully developed, resembles small shark, body opaque, dark bands traversing body, no embryonic movement within the egg case. Yolk stalk becomes thicker.

At 9.5 months

f. Similar in colour to a neonate and almost ready for hatching.

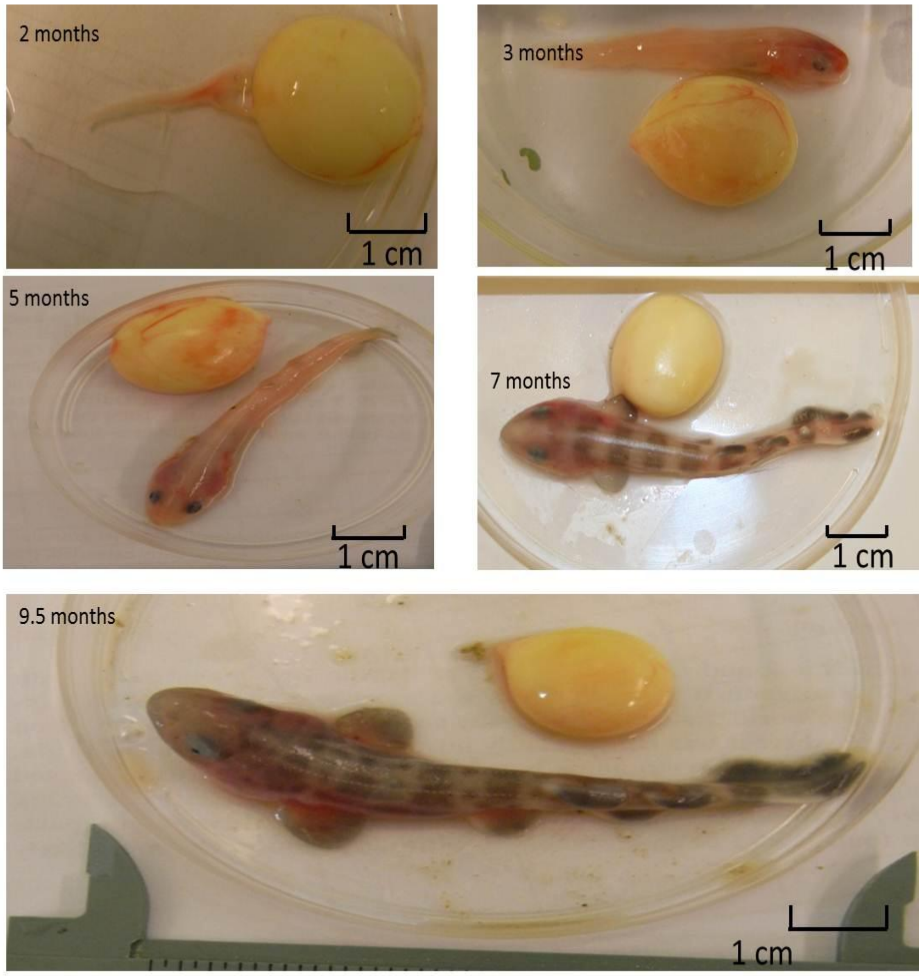

Figure 3.9. Morphological changes of *P. pantherinum* during development.

According to Figure 3.10, the final weight of the hatching embryo was more than the initial weight of the yolk. It is clear that as the embryo mass increased, the yolk mass decreased. The relationship between yolk mass with age was fitted with a negative linear regression. The relationship between embryo mass with age was fitted with a power curve. Thus in 50 days (between 200 and 250 days) the embryo consumed 0.7 g of yolk, yet grew by 2.387 g. The embryo length-weight relationship is represented in Figure 3.11. When the embryo reached a length of 5 cm, it weighed < 1 g.

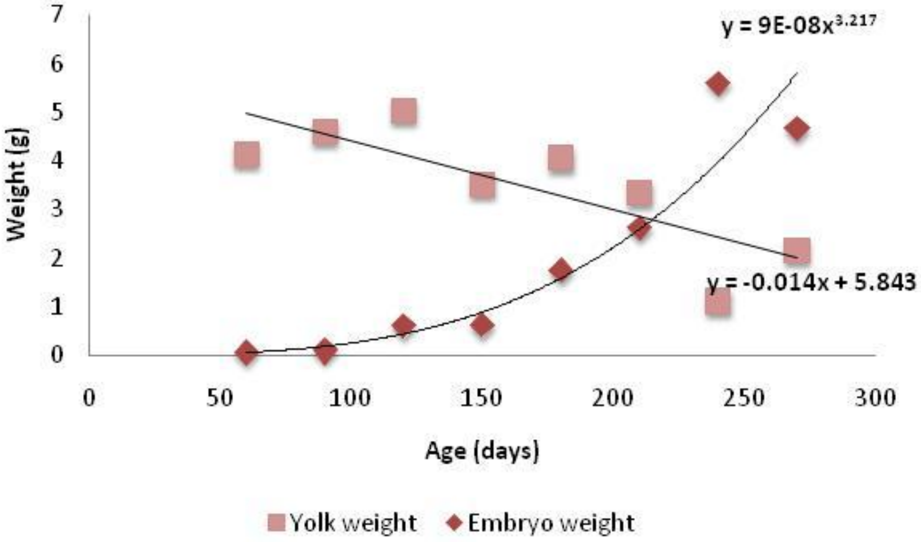

Figure 3.10. Relationships between embryo and yolk weight for *P. pantherinum*.

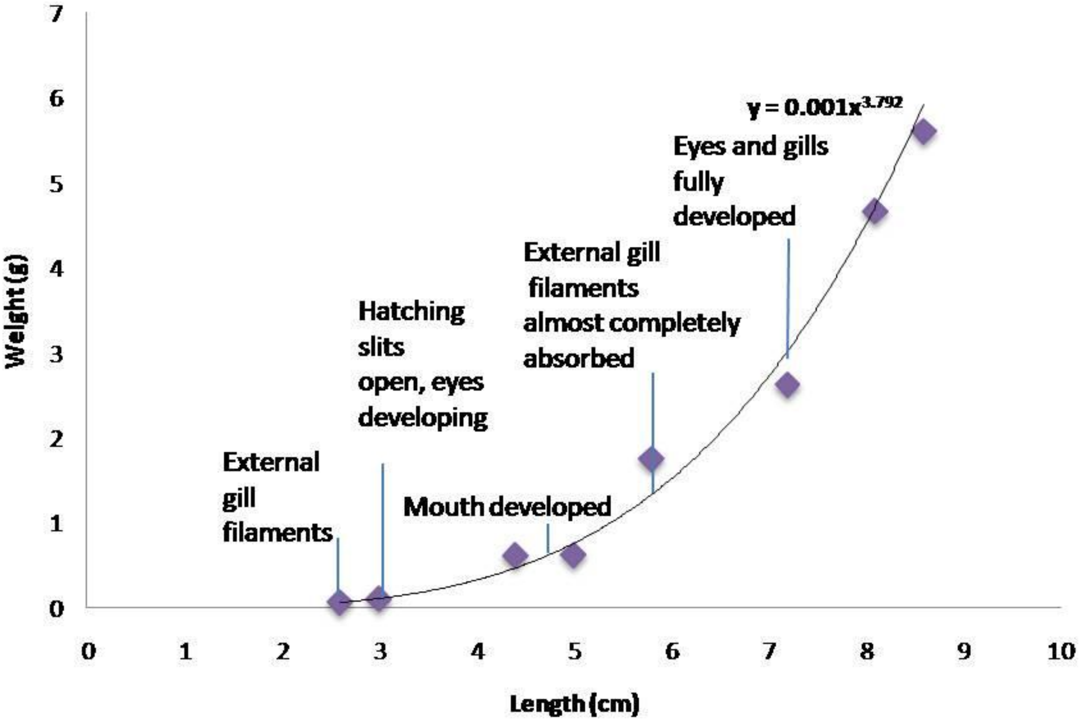

Figure 3.11. Length-weight relationship for *P. pantherinum* during development

Morphological characteristics *of H. pictus* (Figure 3.12)

At 2 Months

a. No eyes, small external gill filaments visible, body opaque, head developing, body thin and elongated, random movement of embryo within egg case.

At 3 months

b. Eyes developing (black pigmentation around eyes), mouth open to the outside, pink notochord running the length of the body, pectoral and anal fins present, both hatching slits open (n=11), yellow gill filaments 0.1 cm, thicker body, embryo movement slower, pulsating, rhythmical.

At 5 months

c. Pink external gill filaments almost fully absorbed, caudal fin undefined in shape, heart developing, colour pigmentation visible on body.

At 6 months

d. Eyes, gills and nostrils fully developed, body resembles a neonate, belly extended by yolk, yolk size drastically reduced, no sign of movement within the egg case, head of embryo facing towards hatching slit.

At 7 months

e. Pigmentation resembles neonate, embryo almost ready for hatching.

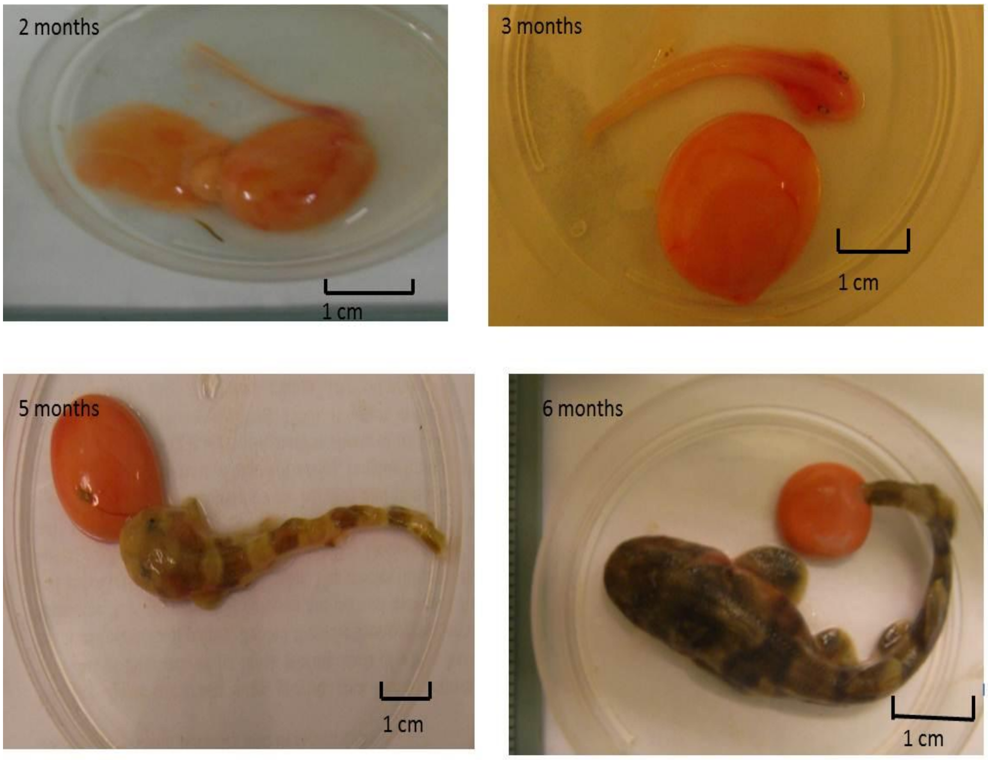

Figure 3.12. Morphological changes of *H. pictus* during development.

The final weight of the juvenile at hatching is greater than the initial weight of the yolk (Figure 3.13). At 60 days the embryo weighed 0.05 g and the yolk mass was 4.18 g. The relationship between embryo mass and age produced a power curve and the relationship between yolk mass with age showed a negative linear regression. When calculating yolk consumption between 150-200 days, the embryo consumed 1 g and grew 2.48 g in 50 days. Figure 3.14 shows an increase in embryo mass with a decrease in yolk mass. The average length of the embryo at 90 days was 3.01 ± 0.83 cm (n=12). It was evident that although embryo length increased, body mass remained constant for 90 days and there was also no change in yolk mass over this period. The average length of the embryo at five months was 5.53 ± 0.7 cm (n=14). By 180 days it had tripled its length (8 cm) and body weight (3 g). The average length at this age was 6.7 ± 0.69 cm (n=7). The decrease in yolk mass at this stage was proportionate to a rapid increase in body weight and length. The average length at 210 days was 8.48 ± 1.3 cm (n=5) and it was at this age that the yolk mass and embryo mass were close to being equal (2.13 g and 3.18 g respectively). At 270 days the yolk weight was 0 g and the embryo weighed 6.7 g. The embryo at this stage was near to hatching. The relationship between embryo length and weight is represented in Figure 3.14.

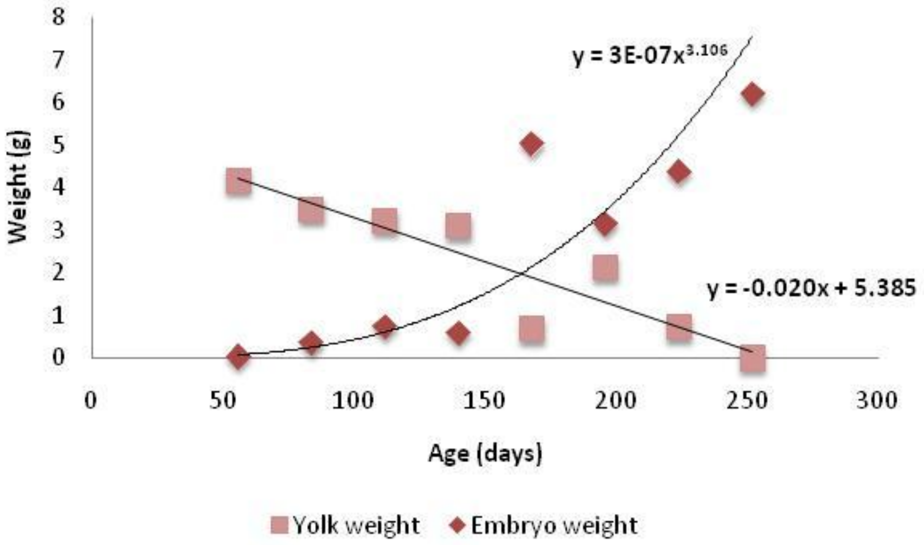

Figure 3.13. Relationships between embryo and yolk weight for *H. pictus.*

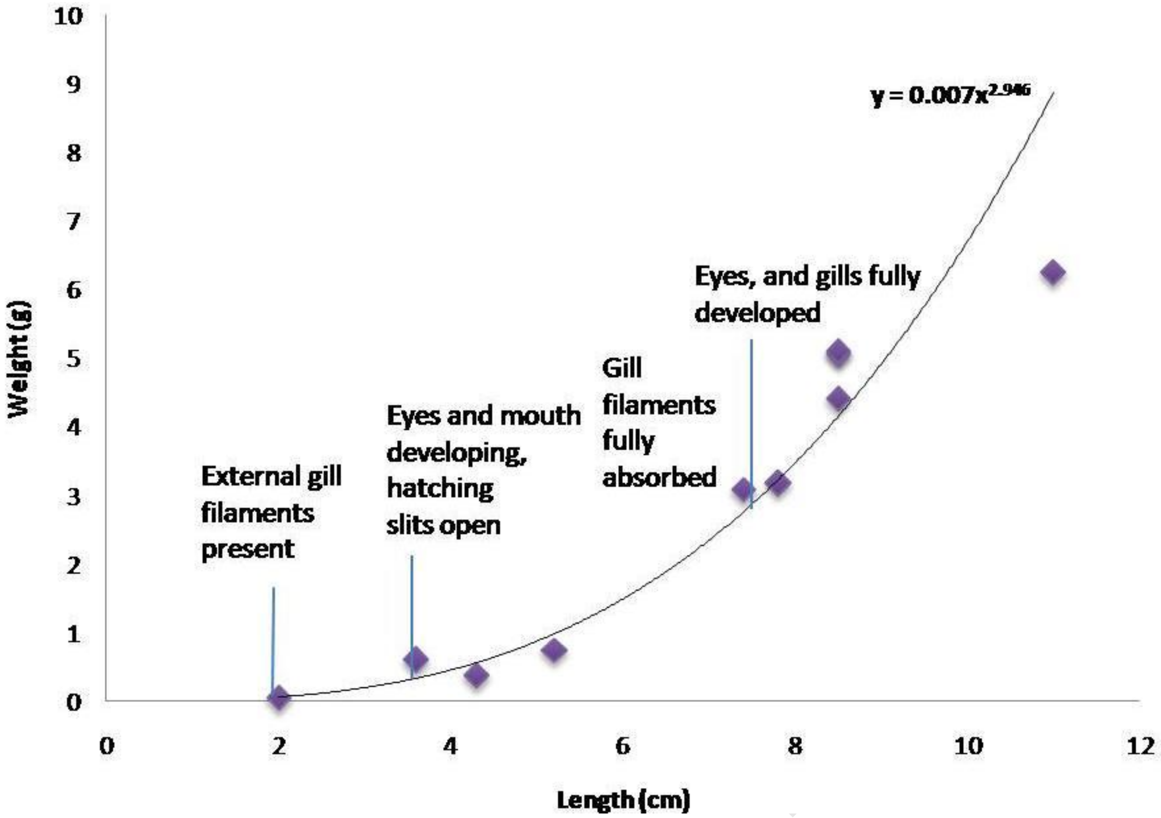

Figure 3.14. Relationship between embryo length and weight for *H. pictus.*

Figure 3.15 refers to a *P. pantherinum* neonate position of the neonate within the egg case just before hatching. Evidence of absorption of the yolk mass was seen in the swollen stomach and the yolk stalk at this stage was thicker.

Figure 3.15. *Poroderma pantherinum* embryo at 8 months with a thick yolk stalk.

Comparison between experimental and field development

Unfortunately the conditions to which the eggs were exposed in the field could not be controlled, so some eggs were predated on and no data could be collected from these. The longer the egg was exposed to dangers the more chance there was of mortality (Table 3.2).

Table 3.2. The number of live embryos found for the replicates at 3, 5 and 7 months.

Species	**Months**		
	3	**5**	**7**
P. pantherinum embryos	3	2	1
H. pictus embryos	4	2	1

Table 3.3 shows a comparison in lengths between both species in the field and the laboratory. It is evident that both species reached similar lengths in the field at three months (3.7 ± 0.92 cm) and seven months (10.2 cm for *P. pantherinum* and 10 cm for *H. pictus*). The lengths were also slightly longer to those of the embryos developing in the laboratory. Table 3.4 shows that the rate of development for both species was the same in the field as in the laboratory at 14°C (0.05 cm/day).

Table 3.3. Comparison between average lengths of *P. pantherinum* and *H. pictus* embryos in the field and laboratory

Age (months)	*P. pantherinum* length **Field (cm)**	 **Lab (cm)**	*H. pictus* length **Field (cm)**	 **Lab (cm)**
3	**3.7 ± 0.92 (n=3)**	**2.5 ± 0.89 (n=15)**	**3.7 ± 0.42 (n=4)**	**3.01 ± 0.83 (n=12)**
5	**6.89 ± 0.58 (n=2)**	**5.1 ± 0.65 (n=11)**	**7.8 ± 0.84 (n=2)**	**5.53 ± 0.7 (n=14)**
7	**10.2 (n=1)**	**7.67 ± 1.19 (n=3)**	**10 (n=1)**	**7.6 ± 1.2 (n=6)**

Table 3.4. Comparison between rates of development in the laboratory and in the field (cm/day).

Species	**14°C Turb**	**14° Still**	**17°C Turb**	**17°C Still**	**Field**
P. pantherinum	0.05	0.05	0.07	0.06	0.05
H. pictus	0.04	0.05	0.09	0.07	0.05

The average daily temperatures were recorded at the site where the experiments took place over a period of seven months. At Simons Town Harbour, the temperature at 10 m varied erratically from 11°C to 16°C (Figure 3.16). The maximum temperature reached was 18°C but then there was a sudden drop to 14°C. These changes in temperatures occurred in only a few days, indicating that the embryos were exposed to rapid fluctuations in temperature. The temperature can change by as much as 4 °C over a few days. As summer approached, the temperature became warmer (15°C) and the fluctuations were smaller. The minimum temperature reached in Glencairn at 2 m was 12°C and the maximum was 19°C (Figure 3.17). There was a gradual increase in temperature as the summer months approached. At one point there was a change in temperature by as much as 3°C, however, the fluctuations were more gradual and less significant than at 10 m.

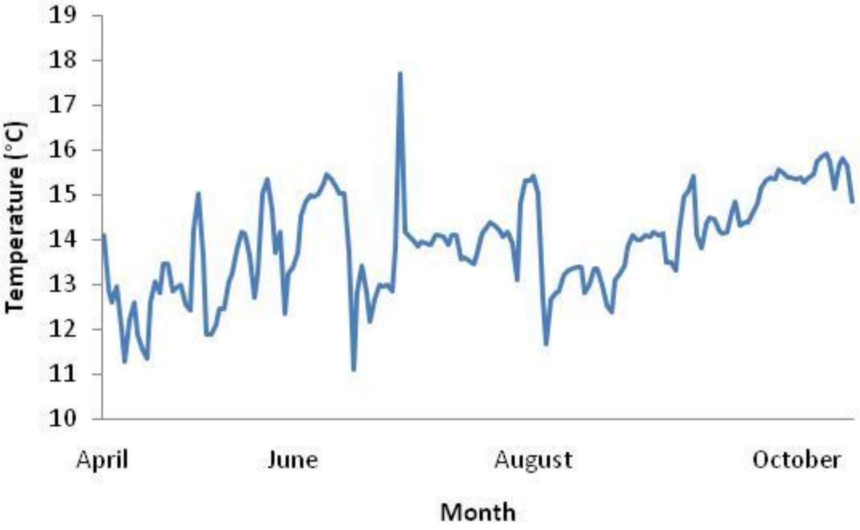

Figure 3.16. Average daily temperatures in Simons Town Harbour over the seven month study period

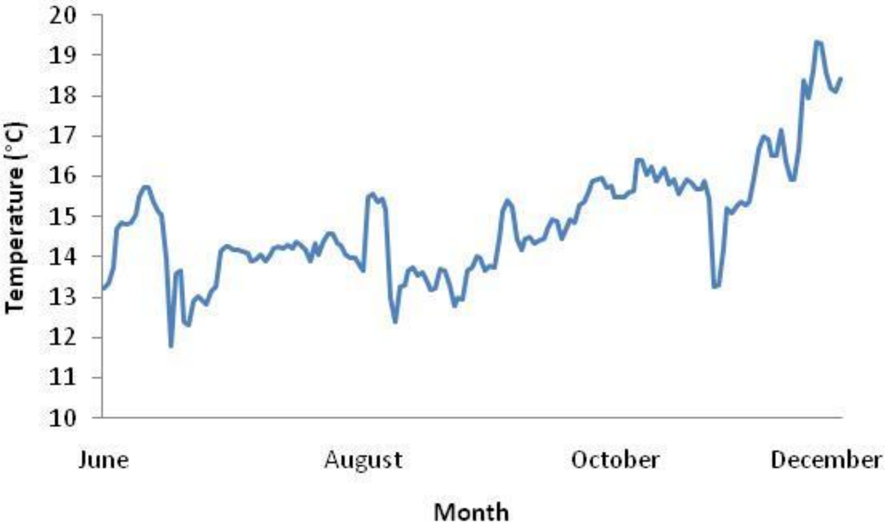

Figure 3.17. Average daily temperatures in Glencairn over the seven month study period.

Average size of juveniles at hatching

The biological data for juveniles collected after hatching are presented in Table 3.5. The condition of the water (turbulent/still) resulted in no significant difference in weight for the neonates of either species (F=1.7; p=0.5). The average lengths of the juveniles for both species were very similar at between 10.66 and 11 cm, but the average weight of juveniles at hatching varied by as much as 3 g in relation to the respective environments to which they were exposed. In turbulent 17 °C water, both *P. pantherinum* and *H. pictus* juveniles had the highest mass of 9 g and 8.19 g respectively (Table 3.5). The finding that juveniles took about 20 weeks longer (*P. pantherinum*), or about six weeks (*H. pictus*) longer to hatch at 14°C only applies to still conditions. Temperature therefore also significantly influences the incubation period (F=30.52; p=0.005).

Table 3.5. Length, weight and incubation period for hatched juveniles

Species and temperature	**Weight (g)**		**Length (cm)**		**Incubation period (weeks)**	
	Turbulent	Still	Turbulent	Still	Turbulent	Still
Temp (°C)	14	14	14	14	14	14
P. pantherinum	*7.33*	*9*	*10.66*	*11*	*40.33*	*38*
Temp (°C)	17	17	17	17	17	17
P. pantherinum	*9*	*8.65*	*11*	*11*	*19*	*25*
Temp (°C)	14	14	14	14	14	14
H. pictus	*6.17*	*6.71*	*10.97*	*10.86*	*30.6*	*31*
Temp (°C)	17	17	17	17	17	17
H. pictus	*8.19*	*7.27*	*11*	*11*	*29*	*25*

Discussion

The effect of temperature and turbulence on the rate of development for P. pantherinum and H. pictus

The length age relationships for both species were linear, as recorded by Castro *et al.* (1988) for *Scyliorhinus retifer* embryos. This is different to the development of *Heterodonotus portusjacksoni,* which grows exponentially and then plateaus near hatching (Rodda, 2000). The development time for *P. pantherinum* embryos was two months longer than that for *H. pictus* embryos (217 days) at 14 °C. Incubation period for *H. portusjacksoni* was strongly temperature-dependant (Rodda, 2000), which explains why development of *P. pantherinum* embryos took virtually half the time and *H. pictus* embryos took at least five weeks less to hatch in warmer water (17°C). Turbulent conditions resulted in a slightly longer incubation period. Embryos exposed to supplemented oxygen thus have an extended incubation period (Latham and Just, 1989; Seymour *et al*., 2000; Czerkies *et al.,* 2001).

Poroderma pantherinum embryos grew 1.4 times faster and *H. pictus* embryos grew 1.8 times faster in a temperature raised by just 3°C. This is very similar to the findings of Harris (1950) who documented the rate of development of *Scyliorhinus canicula* at two different temperatures, showing that the rate of development was 1.64 times longer at a cooler temperature of 11.5°C, as opposed to a warmer temperature of 15.5°C. However, for *P. pantherinum* embryos, an additional 150 days was needed in cooler water for the embryo to reach full development, while *H. pictus* needed an additional 35 days. *H. portusjacksoni* embryos took 291 days to develop at a temperature of 22°C (Rodda, 2000). *H. portusjacksoni* and *P. pantherinum* have similar incubation periods, but at different temperatures (22°C and 14°C respectively). One would expect *H. portusjacksoni* to develop faster at such a high temperature, but due to the size of the embryo at hatching (22 cm as opposed to 11 cm *for P. pantherinum*) more energy is needed to convert the yolk to body mass. The growth rate for both *H. pictus* and *P. pantherinum* were similar (0.05 cm/day) at the cooler temperature of 14°C. A change in temperature resulted in a change in growth rate which was more evident in *H. pictus,* where it almost doubled (0.09 cm/day). This provides evidence that *H. pictus* embryos are sensitive to changes in temperature. Natural oxygen concentrations in the ocean are mainly regulated by salinity and temperature (SA water quality guidelines, 1996). Gaseous exchange occurs at the surface, where air and water meet, and is strengthened through wave action. The concentration and dispersion of oxygen is dependant on its solubility and interaction with other processes of a biological nature (Drew, 1999). Turbulence can thus be defined as an enhancement of gaseous exchange at the surface due to interaction with the atmosphere and increased wave action. Statistically, turbulent and still conditions had little effect on development; however, the growth rate was slightly higher for both species in warm, turbulent water. This is because warmer water has less oxygen, but if oxygen is supplemented to warmer water, it provides optimal conditions for development, thus increasing the growth rate.

Hatching success

The mortality rate for both species was higher at 17°C than at 14°C. The embryos of marine fishes are particularly sensitive during the early stages of development, due to temperature affecting enzymes, which increases their metabolic rate (Lillie and Knowlton, 1897). There is a temperature range at which enzymes function optimally and when the temperature falls out of the optimal range, the possibility exists that it could lead to a decrease in the functionality of the enzyme process, resulting in mortality. Laurence and Rogers (1976) came to the conclusion that temperature (as well as salinity) is the main environmental variable determining embryonic growth and survival. As a result of a few mishaps in the aquarium (pumps breaking), the temperature range fell beyond the optimal temperature range for development, resulting in a high mortality rate. The mortality rate was higher for *P. pantherinum,* indicating that the enzymes of these species could not tolerate such high temperatures (24°C). This could possibly explain why eggs in the wild are predominantly found in deeper waters, where the embryo is exposed to cooler waters and not in shallow waters, where it is warmer due to direct heat from the sun. *Haploblepharus pictus* embryos are found in shallow waters, as they can tolerate warmer temperatures. The mortality rate was also higher in comparison to *H. pictus* at 14°C. This could also be due to the artificial environment in which they were growing not meeting the water quality requirements found in the field beyond 10 m.

Morphological changes during development for P. pantherinum and H. pictus

Early stages of development were very similar to those described by Smith (1942) for *Heterodontus* and by Castro *et al.* (1988) for *Scyliorhinus*. The development of *P. pantherinum* and *H. pictus* embryos during the first five months of development was very similar in that the development of the eyes in *P. pantherinum* happened a few weeks later. The hatching slits remain closed during the first few months of development (Ebert *et al.,* 2006), but for both species, they opened at the same time (c 60-90 days). The embryo possesses a hatching gland which, upon secretion, dissolves the albumen in the fissures for ventilation, and that in the hatching slit once the embryo is ready for hatching (Ballard *et al.*, 1993). The time it takes for the albumen to dissolve, however, seems to vary amongst species, with *Scyliorhinus canicula* taking *c*70-80 days to open (Ballard *et al*., 1993), and *Heterodontus portusjacksoni c*120 days (Rodda and Seymour, 2008). The opening of the mucous plugs is not temperature dependant. The fissures are then completely open to allow the entry of oxygenated water and to remove metabolic wastes. External gill filaments are one of the first respiratory aids to develop in embryo development (Von Bonde, 1945a). For both *P. pantherinum* and *H. pictus* they develop during the first two months of development. The gill filaments serve a dual purpose, in that they are not only used for respiration, by also for urea retention until the embryo is capable of osmoregulation (Smith, 1936; Read, 1968, Kormanick, 1993). Small gill filaments were still visible 129 days into development for both species. This differs from Von Bonde (1945a) who observed the external gill filaments of *H. pictus* to be fully absorbed after 50 days. Movement of the embryo within the egg case varied at different stages. The embryo for both species was very active during the first few months of development. Limited passive ventilation would require active ventilation by the embryo, which would have metabolic implications for the embryo. Active ventilation by *Apristurus* has been observed by Flammang (2005), where the embryo used its tail to sweep water in through the open fissures to allow for greater oxygen exchange. When the external gill filaments are present, the embryo is very active in the egg case and when the internal gills are fully developed, embryo movement is not observed, due to the more advanced method that is now available for respiration, as well as to conserve the energy for development. Leonard *et al*., (1999) were able to prove that embryonic movements from skates (*Raja erinacea*), which were used for ventilation purposes, increased the standard metabolic rate by approximately 20%. When calculating the change in yolk mass at different stages it is evident that it decreased gradually during the first few months of development. There was a difference in the yolk rate consumption for both species at 14 °C*. Poroderma pantherinum* embryos consumed yolk at a rate of 0.014 g/day, whereas *H. pictus* embryos consumed yolk at 0.021 g/day. This suggests that yolk consumption is a gradual process in *P. pantherinum* embryos, but increases significantly towards the end of development. The increase in the width of the yolk stalk also provides evidence to explain yolk consumption towards the end of development for *P. pantherinum* embryos. By the time *H. pictus* eggs reach nine months, all the yolk had been absorbed, whereas *P. pantherinum* embryos still had 2 g of yolk to absorb. The initial yolk weights and growth rates for both species were very similar, however, due to the shorter incubation period of *H. pictus*, it would result in faster yolk consumption. Unfortunately, the consumption rate for both species could not be calculated at 17°C due to the high mortality rate. One would assume that an increase in temperature would increase the consumption rate, due to an increased growth rate. When determining the change in body mass with embryo development, Tullis and Peterson (2000) discovered that the body mass of Bamboo sharks increases sigmoidally with age and that the yolk mass decreases. For *H. pictus* and *P. pantherinum,* the body mass showed a power curve. Both embryos grew at a mass of 0.65 g/cm *(P. pantherinum*) and 0.66 g/cm (*H.*

pictus) at a temperature of 14°C, even though the yolk consumption rate was greater for *H. pictus*. This again leads back to the shorter incubation period, because hatching takes place when the food reserves (external yolk sac) are depleted (Ballard *et al.*, 1993). During the last month of development, *H. pictus* embryos and *P. pantherinum* embryos were similar in weight.

Comparison between growth in the field and in the laboratory

The growth rate in the field for both species was the same, and also similar to the growth rate in the laboratory at 14°C. However the morphometrics differed in the field when compared to embryos of the same age in the laboratory. There is evidence of fluctuations in temperature at the depths at which these two species develop. Although the fluctuations were not for prolonged periods of time, the embryos were still exposed to changes in temperature of as much as 4°C over a few days, thus potentially affecting the enzymes controlling the metabolic rate (Lillie and Knowlton, 1897). The temperature range to which *H. pictus* embryos were exposed (12 to 19°C) was slightly higher than for *P. pantherinum* (11 to 16°C). This could explain the lower mortality rate of *H. pictus* in the laboratory. The temperature in the laboratory at 14°C was within the optimal range for enzymatic processes (Lillie and Knowlton, 1897). According to Von Bonde (1945a) and Compagno *et al.* (2005), the incubation period for *H. pictus* is 104 days. However, the temperature at which these embryos developed was not documented. The results from the field clearly indicate that the incubation period for *H. pictus* is at least 180 days, as there was still one embryo present at 200 days. According to Compagno *et al.* (2005), the incubation period for *P. pantherinum* is 165 days, however, according to the experimental field results, the incubation period is at least 210 days, as there was still an embryo developing within the egg case when it was recovered from the field experiment. From this information, it is difficult to establish the incubation period due to fluctuations in temperature and exact date of oviposition, as was discovered by Von Bonde in 1945.

Size of juveniles at hatching

The lengths of the juveniles for both species at hatching were similar (11 cm). However, the mass differed for both species according to the temperature to which the embryo was exposed. At cooler temperatures (14°C), the juveniles weighed less (regardless of whether the embryo was exposed to turbulent or still conditions), with the average weight for *P. pantherinum* being 8.16 g and 6.44 g for *H. pictus,* but in warmer turbulent water (17°C), the mass of the juveniles increased to 8.83 g for *P. pantherinum* and 7.73 g for *H. pictus*. These results clearly indicate that the effects of temperature on embryo development are significant as it has the ability to affect the rate of development, mortality, yolk consumption, and weight of juveniles. All these results provide evidence suggesting that temperature is the main variable affecting embryo development and which influences egg laying.

Chapter 4

Fouling of *Poroderma pantherinum* and *Haploblepharus pictus* egg cases

Introduction

Biofouling is a process whereby organisms (epibionts) in an aquatic environment (predominantly marine) colonize a surface. Factors that influence larval settlement include hydrodynamics (Dutman, 1987), light, salinity and temperature (Rodriguez *et al.,* 1993). Shark eggs are a favorable surface for the settlement of epibionts, due to their sedentary nature and continual exposure to seawater. Epibionts can cause subtle yet harmful effects to the host, such as reduced light, changes to pH (Sandjensen, 1977) and more importantly, reduced water flow (Scardino, 2006). As shark eggs possess ventilation slits to allow fresh seawater to enter the egg case during embryo development, and the permeability of the egg case must be maintained to allow for the diffusion of oxygen, epibionts covering the egg case could affect the respiration of the developing embryo. The rate of consumption of oxygen is directly proportional to embryonic development, due to the conversion of relatively inert yolk into metabolically active tissue (Bradford and Seymore, 1988; Booth, 1995; Cohen and Strathmann, 1996; Seymore *et al.,* 2000). Hypoxia therefore has the ability to slow rate of development and can also result in mortality. Past research on the antifouiling performance of *Scyliorhinus canicula* egg cases showed that rapidly-growing filamentous green algae can lead to embryo mortality in high light intensities, possibly due to asphyxiation (Thomason, 1994). This could help explain why female dogfish avoid this type of environment when laying eggs (Davenport *et al.,* 1999).

As a result of the potential negative impacts of fouling, many organisms have developed mechanisms for protection against fouling (Wahl, 1987). Even though there is no direct supporting evidence, some shark specialists support the hypothesis that the ability of elasmobranch eggs to deter fouling is due to the albumen, yolk or embryo secreting an anti--fouling substance (M.H.I Dodd, personal communication). Thomason *et al.* (1994) used slates to compare fouling growth on this inert material, relative to *S. canicula* egg cases under both dim and bright light conditions. Their findings revealed that there was a significant difference between fouling cover on the slates and the egg cases in dim light. The mean percentage cover on the egg cases was 17.4% relative to 58.9% on the fouled slate. Eighty-five percent of the egg cases had less than 15% epibiont cover after continual exposure to seawater for 203 days. By contrast the control slates had 59% (max.) and 24% (min.) mean coverage after only 137 days exposure to flowing seawater. The different groups of fouling organisms that they identified under different light intensities are listed in Table 4.1. Their findings suggested that the embryo played no role in preventing fouling and that the egg cases possessed the anti-fouling mechanism. This anti-fouling defense could either be physical, chemical, or a combination of both (Gerhart *et al.,* 1988). A mechanical defense, however, is unlikely, due to the non-living nature of the egg case. An explanation for the chemical defense could be due to the presence of tannin within the egg case (Rusaouen-Innocent, 1990) that might have the ability to deter organisms (Mitchell and Kirchman, 1984). The smooth texture of the egg case could also act as a physical defense (Bradley *et al.,* 1987; Evans, 1988). According to Thomason *et al*. (1994), heterogenous bacteria exists on the surface of the egg case at an early stage, which promotes microfouling, but the lack of EPS (Exoplysaccharide) producing bacteria reduces the colonization of macrofoulers. Some egg cases, however, did have a high rate of fouling and they argued that it was due to imperfections in the female's secretory processes. Hoff (2007) studied the rate of fouling on Alaskan skate egg cases and discovered that the anti-fouling properties within the egg case reduced after 300 days of exposure to seawater. He hypothesized that the fouling mechanisms of the egg case reduces naturally once the embryo has reached a later stage in its development, when it is no longer vulnerable. After completion of his experiment, he was able to determine that hatched cases (that were exposed to seawater for >300 days) had an average fouling cover of 42% and that it had no significant impact on the rate of embryo development.

Table 4.1. Epibiont species found on dogfish egg cases under different exposure to light by Thomason *et al.* (1994).

	Dim light	High light
Group	**Species**	**Species**
Annelida	*Pomatoceros triqueter* *Spirorbis borealis*	*Pomatoceros triqueter* *Spirorbis borealis*
Cirripedia	*Semibalanus balanoides*	*Semibalanus balanoides*
Bryozoa	*Unidentified*	*Unidentified*
Bivalvia	*Anomia ephippium*	*Mytilus edulis*
Ascidiacea	*Botryllus sp*	*Ciona intestinalis*
Anthozoa		*Metridium senile*
Algae:		
Green		*Ulva intestinalis*
Filamentous	*Ectocarpus sp*	*Ectocarpus sp*
Coralline (red)	*Phymalolithon sp*	
	Melobesia membranacea	
	Titanoderma pustulatum	
Coralline (green)	*Lamouroux*	
	Preophyllum sp	

Embryos of oviparous elasmobranchs are encased within a tough capsule that aids in the protection against predators and other external environmental factors (Kormanik, 1992). However, the developing embryo is still exposed to risks of mortality, especially due to predation (Cox *et al*., 1999). A wide variety of organisms are known to predate on elasmobranch eggs, including teleosts, elasmobranchs, pinnipeds, sperm whales and baboons (Bor and Santos, 2003) with the highest mortalities observed from gastropod molluscs (Cox and Koob, 1991; Smith and Griffiths, 1997; Cox *et al*., 1999, Lucifora and Garcia, 2004). Furthermore, fouling organisms growing on the egg cases may be predated on, thus exposing the host egg to potential grazer damage (Dixon *et al.*, 1981, Oswald *et al.*, 1984, Bronmark, 1985, D’ Antonio 1985). This is yet another negative impact of fouling on shark eggs.

There is no previously published information on fouling growth, or the existence of antifouling properties in *Haploblepharus pictus* or *Poroderma pantherinum* egg cases. The aim of this study was to quantify and compare growth cover on plastic relative to live eggs and empty eggs of each species over time. This could provide further insight into the antifouling mechanisms of elasmobranch egg cases.

Materials and Methods

1. Experimental design for *H. pictus* trials

A total of 24 *H. pictus* eggs were used to conduct this experiment. Half the eggs were cut open and the yolk removed. A live egg, an empty egg case and a piece of plastic the size of an egg case were attached to rope fastened to heavy metal links and deployed at a depth of 3 m in Glencairn (Figure 4.1). Two temperature loggers (Tidbit v2) were fastened to the heavy link. The eggs were harvested at three different ages during development- at three, five and seven months. Any live embryos were removed from the egg case and killed to record growth data, as in Chapter 3. The fouling organisms were then identified under a microscope and the percent cover of each visually estimated.

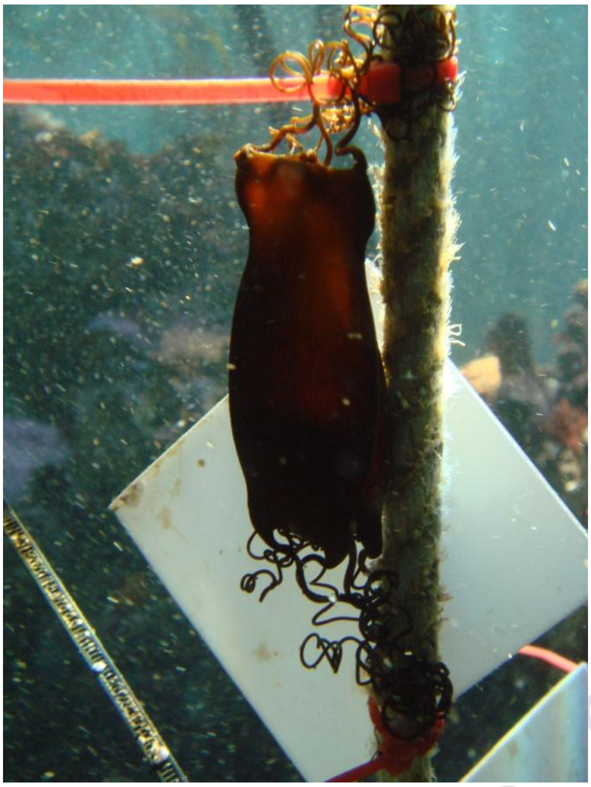

Figure 4.1. Example of an *H. pictus* replicate in the field.

2. Experimental design for *P. pantherinum* trials

A total of 72 *P. pantherinum* eggs were used to conduct this experiment. Half the eggs were cut open and the yolk removed. A live egg, an empty egg case and a piece of plastic the size of an egg case were cable-tied together and placed in the field as a replicate unit. The experiment was conducted at Castle Rocks and 5 -10 replicates were deployed over each of several months as eggs became available. The eggs were fastened to kelp using cable ties. Two temperature loggers (Tidbit v2) were deployed near the eggs to record temperature for the duration of the experiment. This area was chosen as it was more sheltered from storms and minimizing the risk of the eggs being washed away. Another method was explored to ensure that the eggs would remain in place during the duration of development. This was done by taking pieces of rope (1 m) and splicing the tendrils of the egg cases through the rope. Three replicates were placed on each rope, with a float attached above the rope and the rope positioned vertically in the water column (Figure 4.2). Six ropes were deployed at depths of 9-12 m and securely fastened to moorings under the jetty with cable ties. Two temperature loggers (Tidbit v2) were deployed on one mooring.

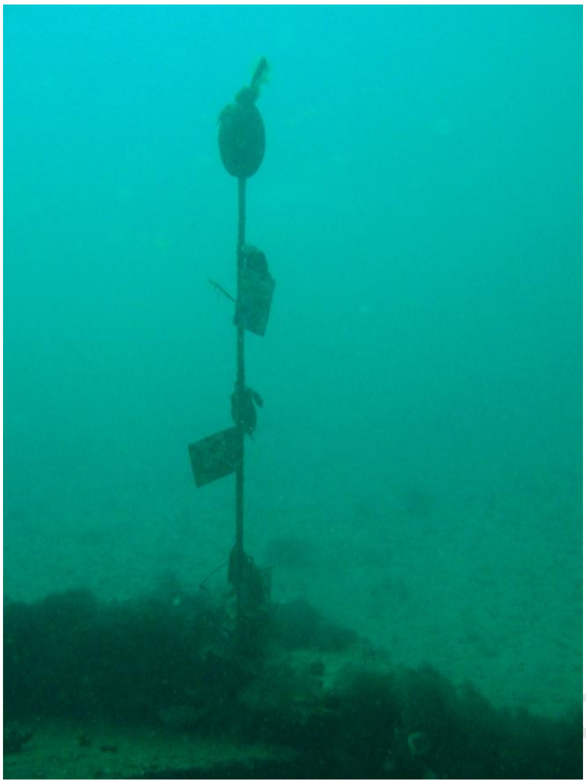

Figure 4.2. *P. pantherinum* replicates in the field.

Statistical Analysis

Mulitfactorial ANOVA was used to test for significant differences between treatments (live egg, empty egg and plastic) and over time.

Results

i. Fouling on live *H. pictus* egg cases

At three months species diversity was low, with encrusting algae (1%), filamentous algae (6%) and Cirripedia (1%), together covering an average of 8% of the egg cases (Table 4.2). At five months, the number of species increased to include Bryozoa (4%), Cirripedia (2%) and Hydrozoa (1%), and the average cover increased to 18% (Figure 4.3). By seven months species from at least seven groups were recorded, with an increase in cover of Bryozoa (20%) and filamentous algae (13%) and an addition of Red/Brown algae (<1%). Cirripedia (1%) and encrusting algae (4%) were still present. Total fouling cover approximately doubled every two months - from 8% at three months, to 18% at five months and 45% at seven months (Figure 4.3).

ii. Fouling on empty *H. pictus* egg cases

Cirripedia (1%), filamentous algae (6%) and encrusting algae (1%) were present at three months and increased in later samples (1-2%) (Table 4.2). A high percentage cover of Bryozoa was observed at five months (9%) increasing to (14%) at seven months. There was a steady increase in growth cover over time (Figure 4.3), a total of 6, 13 and 23% cover being observed at three, five and seven months respectively.

iii. Fouling on plastic

Cirripedia (1%), filamentous algae (12%) and encrusting algae (5%) were the only groups observed at three months (Table 4.2). By five months, the only groups observed were encrusting algae (14%). The total percent cover was similar at three months and five months (15-20%). By seven months, the number of taxa increased to include Ascidiacea (1%), Bryozoa (4%) and Hydrozoa (5%). The total fouling cover at three and five months was <30% and at seven months was 51% (Figure 4.3).

Table 4.2. Percentage cover of different groups of fouling organisms on live and empty egg cases and on plastic over time for *H. pictus* experiments.

Groups/ Species	Substratum and time elapsed (months)								
	Live 3	Live 5	Live 7	Empty 3	Empty 5	Empty 7	Plastic 3	Plastic 5	Plastic 7
Hydrozoa	0	1	2	0	0	6	0	0	5
Annelida	0	0	1	0	0	1	0	0	0
Cirripedia	1	2	1	1	1	2	1	0	0
Bryozoa	0	4	22	0	9	14	0	0	4
Bivalvia	0	0	0	0	0	0	0	0	0
Ascidiacea	0	0	0	0	0	0	0	0	1
Algae (Red)	0	0	<1	0	0	0	0	0	0
Algae (Brown)	0	0	<1	0	0	0	0	0	0
Algae (Filamentous)	6	0	13	5	0	0	12	0	33
Algae (Encrusting)	1	11	4	0	3	0	5	14	8
Total	**8**	**18**	**45**	**6**	**13**	**23**	**18**	**14**	**51**

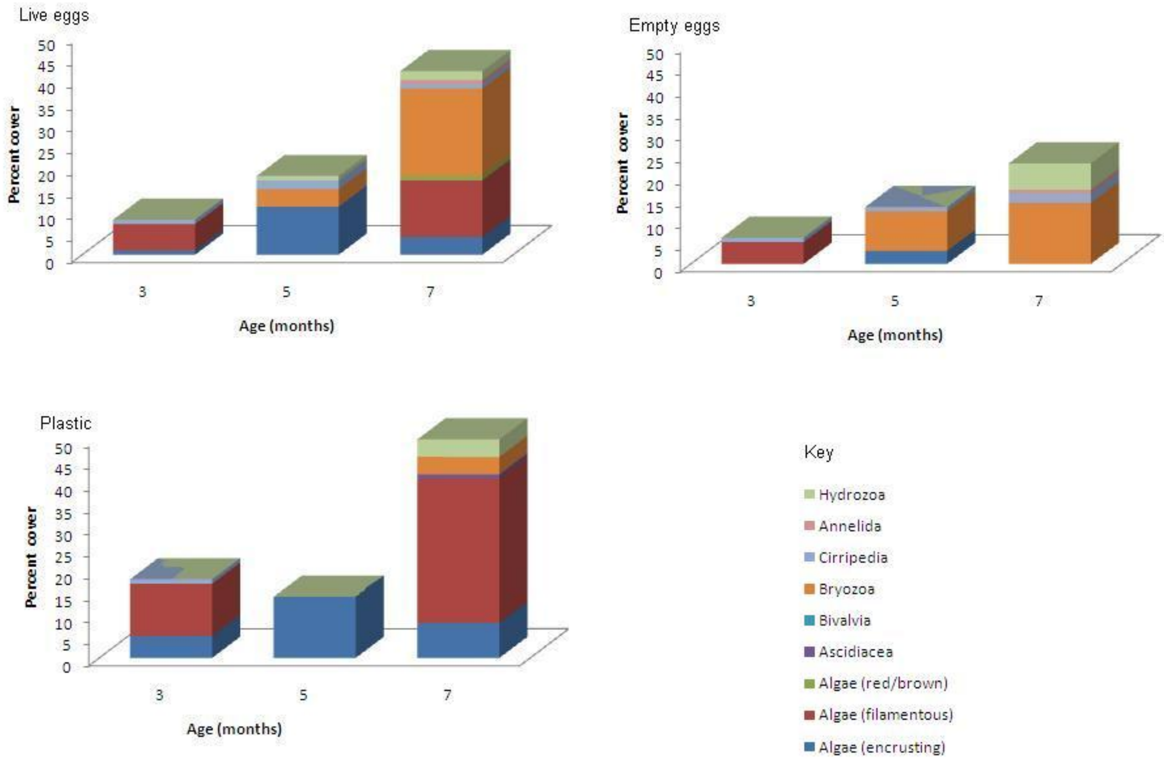

Figure 4.3. Percent fouling cover of various taxa for *H. pictus* replicates over time for live eggs, empty eggs and plastic.

Statistically, there was no difference in percentage cover for the live eggs, empty eggs and plastic (F=2.4; p=0.103), however, there was a significant difference in fouling over time (F= 11.6; p=0.0002).

The trends in average daily temperatures at a fixed location at 3 m are shown in Figure 4.4. Temperatures fluctuated daily, but gradually increased from a minimum temperature in July (11.8°C) to a maximum in December (19.3°C). The average temperature was 14.8°C.

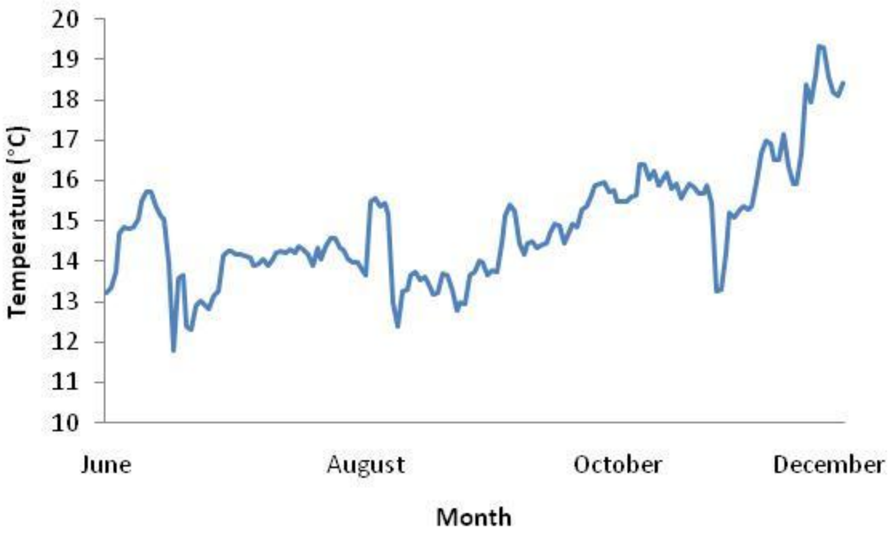

Figure 4.4. Changes in temperature at one location in Glencairn, at 3 m depth.

Predation

There was no evidence of predation of the live eggs on the replicates at three months and all the embryos were still alive. However, at five months two of the four live eggs were predated as evidenced by a small square hole bored by an organism with a radula (Figure 4.5). At this time only one embryo was present. At seven months, three of the four eggs hatched and only one embryo was present.

Figure 4.5. Circle highlighting predation damage on an *H. pictus* egg case at five months. Colonization of Cirripedia, Bryozoa and algae are also evident.

i. Fouling on live *P. pantherinum* egg cases

Annelida and filamentous algae were the only groups found at all three time periods (Table 4.3). There was a correlation between encrusting algae and filamentous algae over time. As encrusting algae decreased (14-8%), filamentous algae increased (14-30%). Ascidiacea (14%) started developing at five months and also increased with time to 21%. The total percent cover increased progressively over time from 33-65% (Figure 4.6).

ii. Fouling on empty *P. pantherinum* egg cases

At three months, encrusting algae and filamentous algae were the dominant groups fouling the egg cases with 18% and 31% cover respectively (Table 4.3). Bryozoa had the next highest cover of 7%. Encrusting algae and filamentous algae were still present at five months, but other groups, such as Ascidiacea (29%) and Cirripedia (6%) were also present. Similarly, all the species present on the egg cases at five months were present at seven months - encrusting algae (8%), Cirripedia (4%), filamentous algae (30%) and Ascidiacea (21%). The total percent cover was similar (>50%) at all time periods, but the species composition differed over time (Figure 4.6).

iii. Fouling on plastic

At three months, encrusting algae and filamentous algae were the dominant groups, with a percent cover of 32% and 19% respectively (Table 4.3). Ascidiacea had the next highest cover of 3%. Encrusting algae (6%) and filamentous algae (6%) decreased at five months, but Ascidiacea increased (19%); a few Annelida (1%) were also observed. At seven months, the percent cover of encrusting algae increased (33%) and the groups increased to include Bryozoa (9%), Bivalvia (1%) and Cirripedia (1%). The total percent cover was similar (>50%) at all time periods, but the species composition differed over time (Figure 4.6).

The difference in fouling percentage cover at three months between live eggs, empty eggs and plastic was 35% and >50% (for both empty and plastic) respectively. The difference, however, was not significant (P=0.43).

Table 4.3. Percentage growth of different groups on live and empty egg cases and plastic over time for *P. pantherinum* replicates.

Groups/ Species	Substratum and time elapsed (months)								
	Live 3	Live 5	Live 7	Empty 3	Empty 5	Empty 7	Plastic 3	Plastic 5	Plastic 7
Hydrozoa	0	0	0	0	0	0	0	0	0
Annelida	1	13	1	1	0	1	0	1	2
Cirrepidia	0	0	4	0	6	4	0	0	0
Bryozoa	4	0	0	7	0	0	0	3	9
Bivalvia	0	0	0	0	1	0	0	0	1
Ascidiacea	0	14	21	1	29	21	3	19	10
Algae (Red)	0	0	<1	0	0	<1	0	0	<1
Algae (Filamentous)	14	17	30	31	6	30	19	18	7
Algae (Encrusting)	14	4	8	18	6	8	32	12	33
Total	**33**	**48**	**65**	**58**	**48**	**65**	**54**	**53**	**63**

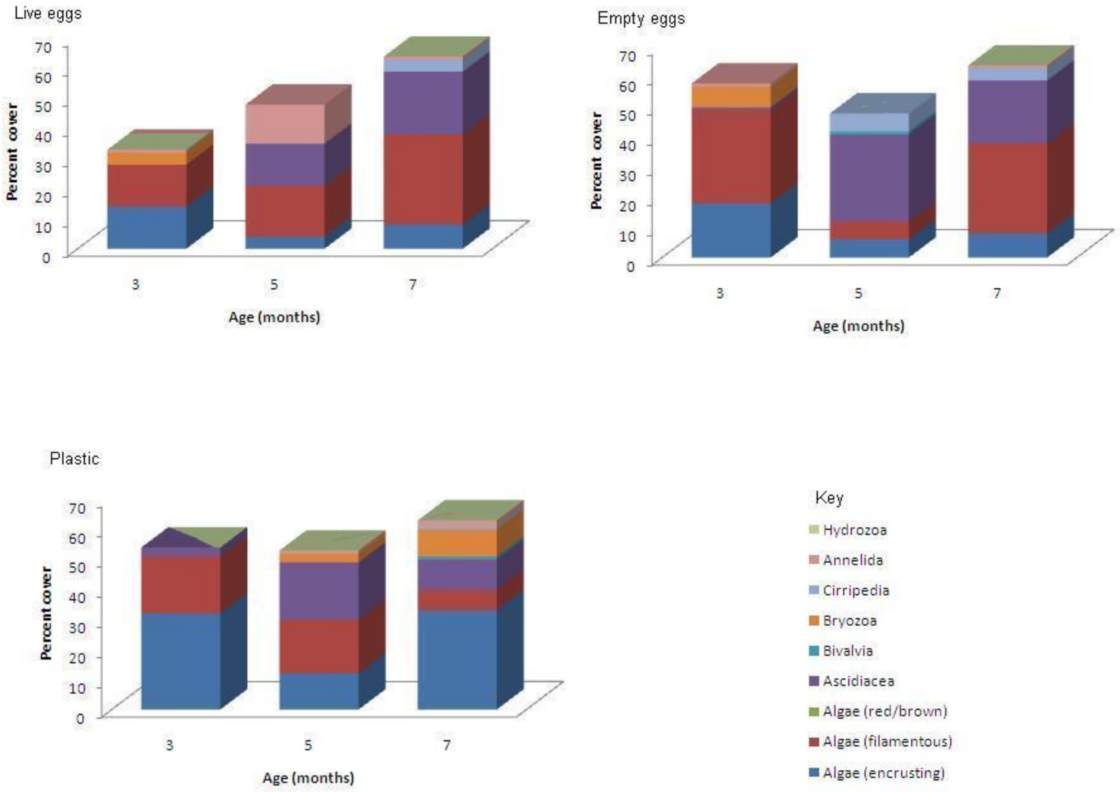

Figure 4.6. Percent fouling cover of various taxa for *P. pantherinum* replicates over time for live eggs, empty eggs and plastic.

Statistically, there was no difference in percentage cover for the live eggs, empty eggs and plastic (F=0.83; p=0.43), however, there was a significant difference in fouling over time (F=3.49; p=0.03).

The trends in average daily temperatures at 10 m during the study period are shown in Figure 4.7. Average daily temperatures gradually increased from June (11.10°C) to October although the maximum was in fact recorded during a brief warm spell in July (17.81°C). The average temperature was 14°C.

The different species identified at the different depths are recorded in Table 4.4. Encrusting algae and filamentous algae and Cirripedia were the common taxa found at both 3 m and 10 m. However, there was greater species diversity at a depth of 10 m. Bryozoa werecommonly found at 3 m and Ascidiacea, Annelida and Bivalvia were commonly found at

10 m.

Table 4.4. Comparison of fouling species found on the two species of egg cases at different depths over time.

Species	**Group**	***H. pictus* at 3 m**			***P. pantherinum* at 10 m**		
		3 months	5 months	7 months	3 months	5 months	7 months
Anomea spp	Bivalvia					√	√
Botryllus	Ascidiacea				√		
Bugula	Ascidiacea				√		
Cerreporella	Bryozoa		√				
Chaetomorpha	Algae (green)	√	√	√	√	√	√
Champia compressa	Algae (red)			√			
Chaperia	Bryozoa		√				
Diplosoma listerianum	Ascidiacea						√
Encrusting algae	Aglae (red/green)	√	√	√	√	√	√
Escharoides	Bryozoa		√				
Kallymaenia schizophylla	Algae (red)						√
Mytilus galloprovincialis	Bivalvia					√	
Notomegabalanus algicola	Crustacea	√	√	√		√	√
Obelia dichotoma	Hydrozoa	√				√	√
Pedicellina	Hydrozoa				√		
Ralfsia	Agae (brown)				√		
Spirorbis	Annelida			√	√	√	√
Unidentified ascidians	Ascidiacea			√	√	√	
Unidentified bryozoans	Bryozoa	√		√			√

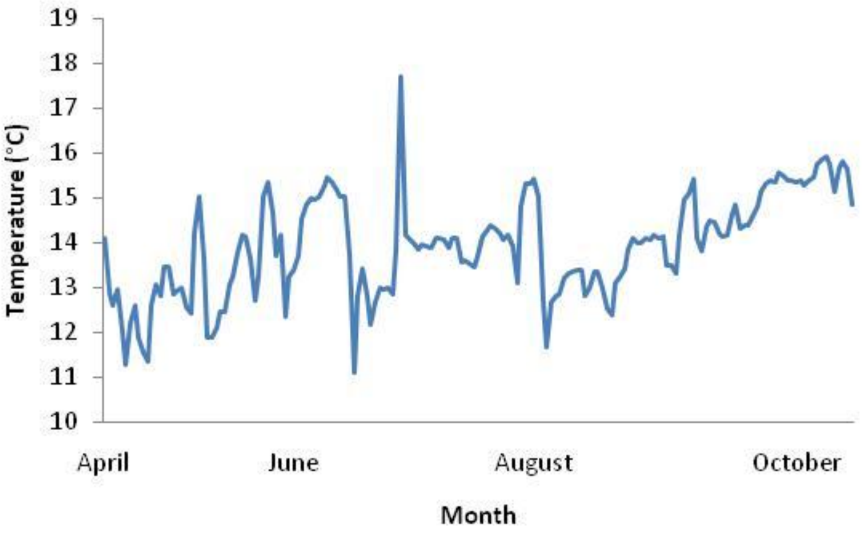

Figure 4.7. Temperature changes at a fixed location in Simons Town Harbour at 10 m.

Predation

There were three embryos still present in the live eggs at three months, however, six of the egg cases were empty. At five months, large holes were visible on six of the egg cases, showing evidence of predation (Figure 4.8). A large number of large black isopods *Paridotea ungulata* were observed in the area over this period and it was speculated that these were the predators (Figure 4.8). Only one of the nine live eggs contained an embryo and the rest were empty. At seven months, four were predated, four hatched and one egg case still contained a developing embryo.

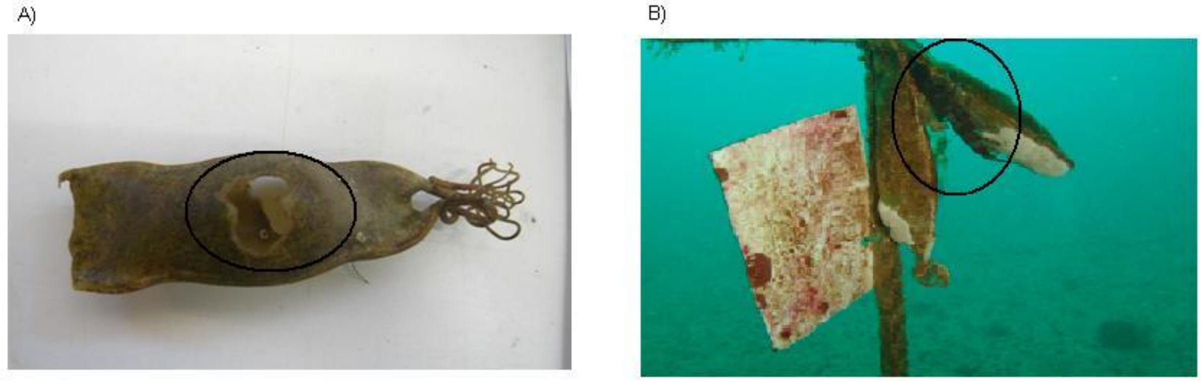

Figure 4.8. A circle highlighting evidence of predation on *P. pantherinum* replicates. B. A circle highlighting the possible predator *Paridotea ungulata* on one of the replicates.

Discussion

Haploblepharus pictus experiment

There was a difference in total cover of fouling between live eggs, dead eggs and plastic at three months (10%, 7% and 20% respectively) in this experiment, with plastic the most fouled. This suggests that the egg case, or the embryo, possesses an anti-fouling property. The main species colonizing the live egg cases at an early stage were filamentous algae and encrusting algae, followed by Bryozoa. This suggests that macro fouling by algae does develop rapidly under light conditions (Thomason *et al.,* 1994). The eggs were placed in very shallow water (3 m), where *H. pictus* females commonly lay their eggs, thus exposing the eggs to high light intensities. The choice of substrata to which the females attach their eggs at these depths (Chapter 2) could assist in shading the eggs from direct sunlight and prevent the attachment of fouling organisms (Dyrynda, 1985). At five months and seven months, the total percent cover was similar on the eggs and plastic (<20% and <70% respectively, but there was a dramatic increase over time. There was a difference in percentage cover observed between the different substrata at three months, suggesting that any anti-fouling properties present in newly laid eggs lose their effectiveness by an age of five months. This was similar to findings of *Scyliorhinus canicula* egg cases that could deter macrofouling for up to six months (Scardino, 2006) and Alaskan skate egg cases up to ten months (Hoff, 2007). At seven months, the total percentage cover on plastic, live eggs and empty eggs were similar (70%). There was therefore no significant difference in total percentage cover at this age. An average of 45% of the live egg case was fouled after 210 days exposure to flowing seawater. Similarly, Alaskan skate egg cases had a total percentage cover of 42% after 300 days exposure to seawater (Hoff, 2007). The embryos of both these species had already hatched by this time. There was no sign of predation and the respiratory seams were open (Smith and Griffiths, 1997).

Poroderma pantherinum experiment

The difference in fouling percentage cover at three months between live eggs, empty eggs and plastic was 35% and >50% (for both empty and plastic) respectively, indicating that the hypothesis could possibly be supported that the embryo possess antifouling properties. Live embryos were still present within the egg case. The inside walls of the live empty eggs were a dark yellow, suggesting a decay of the yolk inside (Rodda, 2000). Similar to *H. pictus*, rapid macrofouling of algae was observed on the surface of the different substrata (Thomason *et al.,* 1994). At five months, the eggs and plastic showed similar fouling cover, even though live embryos were still present within the egg cases. These results were similar to *H. pictus,* also suggesting that *P. pantherinum* egg cases deter fouling up to three months before losing their effectiveness. At seven months, a comparison between the eggs and plastic showed no significant difference in percentage cover between the different substrata, although there was an increase in percentage cover over time.

Comparison between species:

The different environments in which the *Haploblepharus* and *Poroderma* egg cases were placed could help explain the differences in growth cover recorded in the two experiments. The high light intensity and warmer temperatures at 3 m would result in the settlement of different epibionts (Rodriguez *et al.,* 1993; Thomassen *et al.,* 1994).

The fine coating of filamentous algae that commonly develops on egg capsules attracts grazers (Dixon *et al*., 1981, Oswald *et al.,* 1984, Bronmark, 1985, D' Antonio 1985). Algae and Bryozoa are favourable food sources for some marine animals, thus making the egg cases susceptible to predators. However, deliberate predation of the yolk was evident due to holes found on the egg cases. Smith and Griffiths (1997) identified five possible predators of Scyliorhinid egg cases. This observation suggests that a gastropod mollusk must have been responsible for the mortality of *H. pictus* egg cases, as the holes were recorded as being circular. Gastropods are known to be common predators of elasmobranch egg cases (Cox and Koob, 1991; Smith and Griffiths, 1997; Cox *et al*., 1999, Lucifora and Garcia, 2004) and for *P. pantherinum* it was *Paridotea ungulata*. However, this may not be a true reflection of reality in the wild, as the type of substratum on which the eggs are laid can deter both predators and fouling (Dyrynda, 1985).

The total percentage fouling cover on live *H. pictus* egg cases was substantially lower than on live *P. pantherinum* egg cases. This could help explain why the incubation period for *P. pantherinum* embryos was shorter in the field than in the laboratory (Chapter 3). Either the high percentage growth cover induced hatching of the embryos (Petranka *et al.* 1982), or the shorter incubation period was due to the significant changes in temperature (Rodda and Seymour, 2008) at the depth of 10 m (Temperatures in the laboratory remained constant at 14°C, however, temperatures in the field fluctuated between 11°C and 17°C). The incubation period for *H. pictus* embryos were observed to be similar to that in the laboratory (Chapter 3) and this could be as a result of less significant changes in temperature at this depth and less percentage growth cover on the egg cases. With less than half the egg covered by fouling the rate of oxygen supply was probably not being compromised, which would induce hatching (Petranka *et al*., 1982). Hatching is, therefore more than likely induced due to depletion of food reserves (Ballard *et al.*, 1993).

The results for *H. pictus* suggest that the embryo or the egg case may possess antifouling properties, as suggested for *S. canicular* by Thomason *et al.* (1994). However, the results for *P. pantherinum* suggest that the embryo has the ability to deter fouling, supporting the hypothesis that the embryo itself possesses an anti-fouling mechanism.

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