

Several Ecological, Morphological
and Behavioural Aspects of
Territorial Males of
Parablennius incognitus.

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* ABSTRACT *

In this research, several ecological, morphological and ethological aspects of *Parablennius incognitus* males, which are territorial between May and August, were studied. Nest sites of territorial males are cavities in rocks and oscula of sponges, between depths of 0.5 and 4.8 m. These holes are situated in sunlit places not highly exposed to waves and are usually surrounded by a low vegetation of algae. The hole entrance (usually round) generally lies higher than the end of the hole. Minimum entrance diameter varies between 0.73 and 1.68 cm, maximum diameter between 0.73 and 4.27 cm, hole depth between 1.54 and 5.87 cm and hole volume between 2 and 20 ml (respective means are 1.13 cm, 1.56 cm, 3.24 cm and 6.25 ml).

Most territorial *B. incognitus* males leave their natural hole during July. Glass test-tubes, offered as artificial holes, are occupied by *Blennius* males from May onwards and are deserted by the end of August. Most *B. incognitus* males start to occupy a tube between late May and early June; this may depend on the water temperature, which is $\pm 16^{\circ}\text{C}$ during that time. Males occupy tubes at depths of 0.4, 0.9 and 1.6 m, but not at 2.1 m. *B. sphinx* mostly occupies large sized tubes at the former two depths, whilst *B. incognitus* occupies more large than small sized test-tubes with increasing depth.

By the end of August, territorial males are 3.7-5.0 cm in length, weigh 0.49-1.27 g and have gonads of 0.003-0.008 g (respective means are 4.3 cm, 0.85 g and 0.0047 g). At the end of the season, smaller (lighter) males tend to have relatively heavy gonads compared to larger (heavier) males.

Non-social activities displayed by *B. incognitus*, such as the manner of swimming, feeding, scratching and resting, are common to other blennies. Social behaviours, such as agonistic and courtship display, interspecific and intraspecific interaction, are more specific. Territorial males spend most of the day at rest (outwardly), followed by being inside their hole and by ventilating the nest (with eggs) using lateral fins; these behaviours are displayed in similar amounts of time throughout the day (as is interspecific interaction). Sexual behaviours and agonistic interactions mostly take place before noon; swimming, feeding and scratching occur most after noon. This species is diurnal, retreating into holes during the evening to rest during the dark period.

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* INTRODUCTION *

Brief history of the Mediterranean Sea and its fauna

Until the late Miocene, the Mediterranean Sea was a part of the great primordial ocean called the Tethys, which stretched from India-to-be to America-to-be. Some 6 million years ago, the Mediterranean was isolated from the Atlantic and Indian Oceans and became a desiccated sea basin for the first time. During a few million years represented by the Messinian Stage, the Strait of Gibraltar apparently opened and closed several (8-10) times, so that the Mediterranean basin dried up and was re-filled repeatedly (Hsü et al., 1973).

The desiccation of the Mediterranean led to the total destruction of its marine fauna. The present fauna of the Mediterranean Sea descends from Atlantic species, which populated the sea basin at the beginning of the Pliocene when Atlantic waters irreparably broke through the Strait of Gibraltar. During the Pliocene, the strait gradually shoaled eventually cutting off the supply of cold, deep Atlantic waters (Hsü, 1974).

During the glacial periods of the Pleistocene great changes in climate occurred. During warm interglacial periods, water entered the Mediterranean through the Strait of Gibraltar permitting the immigration of thermophilic species. These species died out during the cold periods of time. After the last glacial period, about 10 000 years ago, the water temperature rose and a large number of thermophilic species occupied the Mediterranean Sea. Speciation took place, resulting in new species which are now exclusively found in the Mediterranean and thus are endemic to the region. At present, more than 70 species of fish are endemic to the Mediterranean (Videler, 1980).

The *Blenniidae*

The Mediterranean Sea hosts a rich blennioid fauna represented by the *Blenniidae*, the *Clinidae* and the *Tripterygiidae* (having 1, 2 and 3 dorsal fins respectively).

The first representatives of the suborder *Blennioidei* (*Perciformes*, *Pisces*) appeared in the Tethys about 60 million years ago. It is assumed that speciation of the *Blenniidae* took place after the last glacial period, in the Mediterranean Sea itself (sympatric evolution), after their ancestors had immigrated from the West African coast in post-glacial times.

According to Zander (1973), a 10 000 year interval seems realistic for the speciation of the *Blenniidae*; he supposes an ancestor which was tolerant to variations in light and water-movements and also to different degrees of salinity and temperature. Speciation took place after this ancestor had colonised different biotopes (which were not geographically separated) and each of its descendants had adapted themselves to them. Zander supports his assumption by the existence of so-called 'sibling species'; groups of blennies which are clearly different in ecology (i.e. conditions of light, water movement and salinity), yet hardly different in morphology.

In the Mediterranean there are 22 species of *Blenniidae* of which 13 species are endemic. The *Blenniidae* are small fish, the majority being less than 10 cm in total length. They have a slimy skin without scales and possess one long, continuous dorsal fin and one anal fin. Blennies lack a swim-bladder and are confined to living on hard bottoms; they are predominantly found in the littoral zone. The *Blenniidae* are oviparous and the male shows paternal care by guarding the eggs. Their social and spawning behaviour is highly developed (Zander in Whitehead et al.).

The *Blenniidae* have been grouped in several different ways. Tortonese (1964) grouped the blennies according to their preference for a certain habitat. Zander (1973) divided the *Blenniidae* into groups of sibling-species according to their ecological differences. A few years later, Zander (1978) and Bath (1977) classified the *Blenniidae* in different ways, each providing a new taxonomy for this group. Goldschmid et al. (1978) grouped the different blennies according to their different feeding habits, correlating to morphological adaptations of the teeth. Garcia et al. (1987) suggested yet another classification, after having performed karyotypic studies on several *Blennius* species. Thus, up to now, the systematics of the *Blenniidae* are very different and a more accurate taxonomic review of this group is needed. For the sake of simplicity, the old genus name "*Blennius*" is used throughout this research paper.

Biology of *Parablennius incognitus*

Parablennius incognitus (Bath 1968) is also known as *B. inaequalis* (Valenciennes 1836), *B. ponticus* (Slastenenko 1934), *Pictiblennius incognitus* or, for the sake of simplicity, *Blennius incognitus*. It is very common and widespread in the Mediterranean. This endemic species is also locally found in the Black Sea and off the Iberian Peninsula to Morocco and the Canaries (Zander in Whitehead et al.).

Although *B. incognitus* is a very common species, literature is sparse on details of its morphology and covers even less of its ecology and behaviour.

B.incognitus has a basic grey-greenish colouration with 7-9 vertical olive-brown coloured bands. It has tentacles on the nasal openings and above the eyes. Its body length reaches up to 8 cm (Louisy, 1980). Figure 1 depicts a territorial *B.incognitus* male.

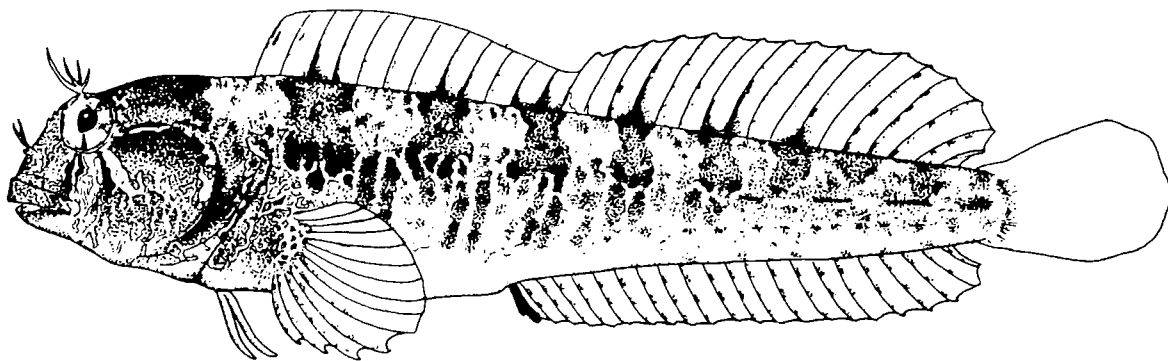


Figure 1. The appearance of a territorial *B.incognitus* male (from Louisy, 1983).

B.incognitus inhabits shallow, rocky littoral areas which are covered with algae and is especially found at vertical walls at depths of 0.5 to 1 m. It is eurybathic, having a broad depth range from 0 to below 4 m. Many other *Blennius* species also inhabit the shallow littoral so that, despite *B.incognitus* having a decreasing abundance from 0.5 to 4 m, its relative (interspecific) abundance may be highest at depths of 2 to 3 m (Illich & Kotrschal, 1990).

B.incognitus grazes the surface of rocky substratum and ingests the inhabiting small benthic animals together with algae and detritus. Sexual dimorphism in feeding habit occurs during the reproductive season, which lasts from May through August. During this time, females feed more on animals whilst males graze the substrate more and sometimes feed on eggs, of the same species (Goldschmid & Kotrschal, 1981).

It is thought that males gain sexual maturity after the first year of life (Kotrschal & Goldschmid, 1981). During the reproductive period, males become highly territorial and each chooses a 'haptic hole' from which it wards off intruders (usually conspecific males). This hole has a diameter which is only slightly larger than the resident male itself. The haptic hole serves as the spawning site for females. This close-fitting shelter affords protection from predators as well as water turbulence and is found in most other endemic *Blennius* species (De Graaf, 1983).

Territorial males (see Figure 1) may be yellow, red, brown, green or olive in colour. Their orbital tentacles become enlarged during the reproductive season and these serve as optic signals to attract females for spawning. Sexually mature males also have club-like glands at the base of the spiny anal finrays; these may secrete a pheromone attractive to conspecific females. Courting behaviours, such as 'rearing up' and 'quivering', are displayed at the entrance of the hole and also serve as optic signals to approaching females (Zander, 1975).

As territorial males display courtship behaviour, females are attracted to their nest. A female is led into the haptic hole of a resident male in which she usually spawns. The male quivers (fertilises the eggs) during or just after spawning. The eggs (spherical to flattened) are attached to one another and clutches are stuck onto the substrate. The larvae hatch after 7-14 days (depending on the water temperature) and lead an extended pelagic existence (Matarese *et al.*, 1984). Several females may spawn inside the hole of one territorial male, which takes care of the eggs.

Territorial males of several endemic *Blennius* species, which guard a haptic hole, are seen to ventilate their 'close-fitting' nest using their fins (Videler, 1980). Territorial males create a circulation of water around their body, which is (partly) positioned inside the hole, by waving their lateral fins up and down. (Ventilating in *B.sphinx* is described by De Graaf, 1983).

Brown (1964) stated that "animals are expected to be territorial, when the seemingly large costs needed to maintain the territory are outweighed by the benefits gained from that territory". The defense and ventilation of a haptic hole by territorial *B.incognitus* males must have its costs, but the shelter that this hole provides, for the male and the eggs, must outweigh the costs of maintaining such a territory.

OBJECTIVE

Since 1980, the Department of Marine Biology of the University of Groningen has been interested in the speciation of the *Tripterygiidae* and the *Blenniidae* in the Mediterranean. This speciation took place in the Mediterranean itself during a relatively short time interval of 10 000 years and is an example of sympatric evolution.

In order to unravel the question of how the speciation took place, basic biological data on different species is needed. Research has already been done on the ecology, morphology and behaviour of three species of *Tripterygiidae* as well as on one species of *Blenniidae*; *B.sphinx*.

The aim of this research project is to contribute to the knowledge on *B.incognitus*.

The questions addressed:

- What size haptic hole is chosen by territorial *B.incognitus* males and where/how is it situated in the surroundings?
Is preference shown for a certain hole size or location?
- Will *B.incognitus* males (or other *Blennius* species) use an artificial hole (a glass test-tube) as a nest site?
If so, is preference shown for a certain tube size or for a tube situated at a particular depth under the water surface?
- At what time during the year do territorial *B.incognitus* males start occupying a haptic hole?
Is the start of the reproductive season related to the water temperature?
How long do males guard their chosen hole?
At what time during the season do they desert their nest?
- What is the length, weight and relative gonad weight of territorial males at the end of the reproductive season?
- Which behaviours are shown by territorial *B.incognitus* males during the day?
At what time during the day are the different activities displayed?

These ecological, morphological and behavioural aspects of territorial males were studied, to obtain a wide scala of basic biological data on *B.incognitus*. This data may be used in later comparative studies, to come to a better understanding of the speciation of the *Blenniidae* in the Mediterranean Sea.

* MATERIALS AND METHODS *

All research was done at Marine Research Station "STARESO" near Calvi, Corsica, between April 22 and August 28, 1991. SCUBA and snorkel techniques were used to gather data under water, on the biology of *B.incognitus*.

I. NATURAL HOLES

During April and May, the coastline just North and South of the port of Stareso was checked every 2-3 days, between 0 and 7 m, for holes occupied by *B.incognitus* males. During the rest of the research period, the coastline was searched less frequently. Positions of nest sites which were found, were marked by tying a numbered, plastic, air-filled test-tube onto a nearby rock using a piece of string. These marked 'natural holes' were checked every 1, 2 or 3 days (with the exception of 4 longer time intervals during May and June), to see if territorial males occupied ('guarded') their nest at different times during the day. The data obtained on the occupation of these natural holes could be compared with data on artificial haptic holes (glass test-tubes), which were offered throughout the research period.

Several parameters of the marked natural holes, each occupied by a territorial *B.incognitus* male, were measured and taken note of:

- Samples were taken of the most common species of algae and sponges (as seen by the eye) in the immediate area surrounding the hole and these were determined in the laboratory.
- The type of substrate, in which the nest was situated, was recorded.
- The distances (in m) between neighbouring natural holes were measured, using a measuring tape, so that a map of the study area could be made.
- The depth (in m) under the water surface, at which the hole entrance was situated, was measured using a digital depth gauge on a diving computer.
- It was noted whether the hole was situated in a dark or sun-lit location and if it was exposed to waves.
- The orientation of the hole entrance was measured using a compass.
- The minimum and maximum entrance diameter (in cm) of the hole was measured using sliding callipers.
- Also using the callipers, the greatest hole depth (in cm) was measured.

- For determining the hole volume (in ml), a 60 ml plastic syringe was used, which was filled with glycerol (coloured with Alcyan blue). The glycerol was slowly injected into the far end of the hole, with the aid of a plastic tube fastened to the end of the syringe. The injected volume of glycerol was noted as soon as the hole was filled and starting to overflow. This method, resting upon glycerol being denser than sea water, was only used for holes whereby the greater part of the hole volume was situated beneath the hole mouth. For holes of which the opposite was true, vegetable oil was used in the syringe, this being less dense than seawater. The hole volume was measured in duplo, after first having removed the testing liquid with the aid of a second syringe.
- The angle of inclination of the hole was measured (in degrees from the horizontal) using a clinograph fastened to a strong tube, which was inserted into the hole.

Results on the above 'ecological parameters' are described and several frequency distribution graphs have been made. Also, graphs showing correlations between minimum diameter, maximum diameter, hole depth and hole volume have been made and corresponding values of regression coefficient are given.

II. ARTIFICIAL HOLES

Artificial holes were offered as nest sites, to see if *B. incognitus* males (or other *Blennius* species) would occupy such holes during the reproductive season. Holes were drilled into four concrete blocks into which glass test-tubes were fitted. Block I was 40 cm in length and 20 cm in breadth and depth. Blocks II, III and IV were 10 cm longer than block I, but had the same breadth and depth.

Glass test-tubes of two different sizes were offered as artificial haptic holes. Block I contained 1 small and 3 large sized tubes, whilst blocks II, III and IV each contained 4 small and 4 large sized tubes. A small sized tube measured 0.85 cm in diameter, 7.9 mm in length and its corresponding volume was 5.0 ml. A large sized tube measured 1.00 cm in diameter, 9.8 cm in length (from now on, termed 'hole depth'), with a volume of 9.0 ml. The distance between any two test-tubes placed in the blocks varied between 8 and 12 cm. The glass tubes could be removed from the blocks to check for the presence of eggs, by pulling a string which was tightly wrapped around the mouth of each tube.

On May 6, 1991 (day 14 of the research period), the four concrete blocks (containing the artificial holes) were placed amongst large granite boulders, which were covered with a thin layer of algae, just South of Stareso port. Blocks I, II, III and IV were placed at different depths under the water surface; at 0.4, 0.9, 1.6 and 2.1 m respectively. The blocks were placed not more than 2 m apart, the test-tubes lying approximately horizontal and facing \pm South-East.

Table 1. Summarised data on the size and position of the artificial holes (glass test-tubes).

Block	Tube size	Depth (m)	Compass direction (bearing)	Entrance diameter (cm)	Hole depth (cm)	Hole volume (ml)	Angle of inclination (degrees)
I	S	0.4	120	0.85	7.9	5	20
	L	0.4	120	1.00	9.8	9	20
II	S	0.9	120	0.85	7.9	5	10
	L	0.9	120	1.00	9.8	9	10
III	S	1.6	110	0.85	7.9	5	0
	L	1.6	110	1.00	9.8	9	0
IV	S	2.1	110	0.85	7.9	5	10
	L	2.1	110	1.00	9.8	9	10

The information on the blocks and test-tubes has been summarised in Table 1. The blocks were checked every 1-2 days (during May), to see if any of the test-tubes was occupied by a territorial male blenny. Per observation (lasting 5-10 minutes per block) the 'occupant species' of each tube was noted. During June, July and August, the blocks were checked less frequently, as research was directed towards territorial *B.incognitus* males which occupied a natural haptic hole.

By the end of the reproductive season, it was possible to present the data on the occupation of the different sized test-tubes (placed at different depths) in two ways. In the results, the 'levels of occupation' during the course of time are presented in line graphs, whilst data on 'overall occupation' (summarised over the whole research period) is presented in pie charts and bar graphs. To be able to compare results on the latter, the data on block I was compensated for the lower number of small and large sized test-tubes that it contained.

If males of different *Blennius* species occupied test-tubes of a particular size, or at a particular depth, more often than other tubes, it was assumed that they 'preferred' such artificial holes. Such differences (in overall occupation numbers) between species were tested for significance, using the Chi-square test.

To see if a correlation exists between water temperature and the time during the season in which males start to occupy an artificial hole, the water temperature was recorded daily. A thermometer was permanently situated at a depth of ± 2 m under the water surface, against the East side of the quay wall. The temperature (in degrees celsius) was recorded between 10:00 and 12:00 hours, from April 22 (day 0) to August 17 (day 117), 1991; the corresponding data is graphically displayed in the results.

III. MORPHOLOGICAL ASPECTS

August marks the end of the reproductive season of *B. incognitus*. During the last week of this month, the few remaining territorial males, guarding an artificial or a natural hole, were caught (as well as a couple of other males, occupying a shelter in the quay wall). This was done by holding a plastic bag over the hole entrance and agitating the male with a stick. The disturbed male shot out of its hole and was easily caught in the bag. The territorial males were killed in cold sea water that had been refrigerated overnight. This method was preferred, instead of using alcohol or other chemicals, so as to prevent weight changes from occurring due to osmosis.

Several hours later, the total length (in cm) of each individual was measured using a ruler. The territorial males were weighed (to the nearest 0.0001 g), after roll-drying them on one's hand. The males were then dissected under sea water, and the gonads were carefully removed with the aid of a stereoloupe and fine pincers. The gonads were weighed (to the nearest 0.0001 g), after excess water was trailed off on aluminium foil. Gonad weight could be expressed as a percentage of the total weight of the individual (relative gonad weight). The remains of the fish were stored in alcohol so that the otoliths could possibly be used in a later stage to determine the exact age of the fish.

Frequency distribution graphs were made of total length, body weight, gonad weight and relative gonad weight. Values of regression coefficient, for graphs setting body weight against total length, gonad weight against body weight and relative gonad weight against body weight are also given in the results.

IV. BEHAVIOURAL ASPECTS

Observations on behaviour were made while hanging still in the water, about 1 to 3 m in front of marked natural nest sites occupied by *B. incognitus*. This method could be used, as the territorial males seemed to ignore the observer within five minutes of arrival. The study animals were not marked but individual adults could be recognised by the location of their territory, by body size, and by physical characteristics or behaviour.

During the month of May, qualitative observations were made in front of the blocks (which contained the artificial holes), during half-hour periods, at different times during the day. In June, similar qualitative observations were made in front of the natural nest sites. Observation 'rounds' were done to observe territorial males throughout the day. Underwater observations were jotted down on PVC slates, using a pencil.

In this way, several behaviours exhibited by territorial *B.incognitus* males at different times during the day could be recognised and these are described in the results.

In an attempt to gain quantitative data on behaviour, an 'event-recorder' was used during July and August. This underwater instrument can be used to record different activities on a time basis. The event-recorder was specially designed for behavioural work at the University of Groningen. This underwater computer (having 16 keys) is able to record events accurate up to 0.01 sec. The recorded information can be directly transferred into a PC and the data stored. This 'digitalized data' can then be processed by using specially designed programs. For this research, a chronological record of the keys used per observation period could be obtained as well as summarised information per key. Also, time budgets of the various activities, represented by different keys, could be obtained per recorded protocol (string of observations made at one nest).

Between July 5 (day 74) and August 9 (day 109), the event-recorder was used at 14 marked natural nest sites of *B.incognitus*. Underwater observations were 'typed into' the instrument at different times during the day, protocol starting times varying between 06:00 and 21:00 hours. Per protocol, the behaviours displayed by one territorial male were recorded during approximately 10 minutes. If the male was engaged in courting a female or the latter was busy spawning inside the nest, the '10 minutes' were exceeded until such activity had come to an end.

In order to use the event-recorder properly, the different behaviours shown by territorial *B.incognitus* males have been divided into "states" and "events". States are defined as 'long' behaviours, being displayed during a certain period of time. Events are 'short', quick behaviours which almost occur at a certain point in time. One or more events can thus be displayed within certain states.

Each of the following behaviours, displayed by a territorial *B.incognitus* male (unless stated otherwise), could be recorded by using a different key on the event-recorder:

STATES:

- 1 - Swimming
- 2 - Resting (outside or partly inside hole)
- 3 - Ventilating hole using fins (fanning)
- 4 - Agonistic display
- 5 - Courtship display
- 6 - Totally inside hole (active or at rest)
- 7 - Interacting with a different species
- 8 - Interacting with another *B.incognitus* male
- 9 - Interacting with a *B.incognitus* female
- 10 - Interacting with a *B.incognitus* of unknown sex
- 11 - Lost (out of site)

EVENTS:

- 1 - Leaves hole
- 2 - Changes guarding position
- 3 - Trembles (quivers) body
- 4 - Nips vegetation (feeds)
- 5 - Scrapes against substrate (scratches)
- 6 - Female enters hole
- 7 - Female leaves hole

State 11 ('lost') could be registered when a territory owner could no longer be seen (outside of its hole). The duration of this state was subtracted from the duration of the protocol. The event-recorder also possessed a 'correction' key so that accidentally or wrongly recorded keys could be immediately corrected on location.

To be able to compare the display of the different behavioural activities during the day, the protocols were divided into 5 'daytime categories', each category spanning a period of 3 hours. The protocols were divided into these categories according to their 'starting time'; they were not sorted per individual or per date. Data on the states, as well as data on the events, was assimilated per category. Thus, a general picture of 'daily activity' of territorial *B.incognitus* males between July 5 and August 8 could be obtained.

The protocols were divided into the following five daytime categories:

- 06:00 - 09:00 hours: 'early morning'
- 09:00 - 12:00 hours: 'late morning'
- 12:00 - 15:00 hours: 'early afternoon'
- 15:00 - 18:00 hours: 'late afternoon'
- 18:00 - 21:00 hours: 'evening'

Per daytime category, the time spent on each state was calculated as a percentage over the summarised 'true protocol time' of the particular category; true protocol time being the duration of a protocol excluding the duration of the 'start' and 'end' states, as these could not be completely recorded. The data obtained on the states is graphically displayed per activity.

The data obtained on the different events was also assimilated per daytime category. For each of the five categories, the frequency of each event was expressed per hour of the summarised protocol time of the particular 3 hour period. The data gained on the different events is also graphically displayed in the results.

* RESULTS *

I. NATURAL HOLES

SUMMARISED RESULTS

Most (10 of 17 = 58.8 %) territorial *B.incognitus* males stop occupying their nest during July. All 14 marked natural nest sites are deserted by August 8 (day 108). Most (52.9 %) territorial males occupy ('guard') their natural shelter for a period of time between 20 and 45 days. Only 3 (17.6 %) males have an estimated guarding time between 55 and 66 days. Only 1 (5.8 %) male occupies its hole for less than 10 days; this number excludes males which were only seen once or twice.

The 14 marked natural holes or crevices used by territorial *B.incognitus* males, over a period of time during the research period, are found to vary in size and shape as well as in their position to the surrounding area:

- Cavities in granite and concrete rocks, as well as oscula of sponges are used as natural nest sites. The artificial holes were made of glass.
- 'Distance to the nearest conspecific neighbour' varies between 0.35 and 4.8 m, the mean being 1.35 m (s.d.= 1.22); the artificial holes were placed only ± 10 cm apart.
- Depths at which natural holes are situated, range from 0.5 to 4.8 m under the water surface, the mean depth being 1.2 m (s.d.= 1.1). Most (10 = 71.4 %) holes are situated between 0.5 and 1 m. Artificial holes (test-tubes) were offered at depths of 0.4, 0.9, 1.6 and 2.1 m.
- All marked natural nest sites are found in sun-lit places which are not highly exposed to waves. All test-tubes were exposed to sunlight; the deepest holes being least exposed to incoming waves.
- Of 12 natural holes measured, most (7 = 58.3 %) face South-West, having bearings ranging from 180 to 270°. The rest (41.7 %) face different directions. The average bearing is 196.7° (s.d.= 82.3). The artificial test-tubes had bearings between 290 and 300°.
- Minimum entrance diameter varies between 0.73 and 1.68 cm, the mean being 1.13 cm (s.d.= 0.321). Most (10 = 71.4 %) holes have a minimum diameter between 0.7 and 1.2 cm.
- Maximum entrance diameter varies between 0.73 and 4.27 cm, the mean being 1.56 cm (s.d.= 0.997). Most (11 = 78.6 %) holes have a maximum diameter between 0.7 and 1.8 cm. The artificial holes had comparable entrance sizes; diameters were either 0.85 or 1.00 cm.
- Most (10 = 71.4%) of the 14 marked natural holes have approximately round entrances.

- Hole depth varies between 1.54 and 5.87 cm, the average being 3.24 cm (s.d.= 9.70). Most (8 = 57.1 %) holes have a hole depth between 2.5 and 3.5 cm. The artificial holes were deeper, having a hole depth of 7.9 or 9.8 cm.
- Hole volume varies between 2 and 20 ml, the average volume being 6.25 ml (s.d.= 5.35). Most (8 = 57.1 %) holes have a hole volume between 2 and 4 ml. The artificial holes used, had hole volumes (5 and 9 ml) within this range.
- The angle of inclination varies between -80° and $+80^{\circ}$ from the horizontal. Most (11 = 78.6%) holes have an upward slope, having a positive angle of inclination. Artificial holes were placed between 0 and $+10^{\circ}$.
- Maximum entrance diameter is positively correlated to minimum entrance diameter (regression coefficient of +0.47).
- Minimum diameter is positively correlated to hole volume (regression coefficient of +0.43), but only a negligible correlation is found between minimum diameter and hole depth (regression coefficient of +0.07).
- However, hole volume is positively correlated to hole depth (regression coefficient of +0.39).

I.A. OCCUPATION OF NATURAL HOLES

As soon as a *B.incognitus* male was found to occupy a natural hole, the nest site was marked and checked regularly so that the occupation of the site during the rest of the season could be observed. Although the same part of the coastline was checked frequently, a suitable natural hole could only first be noticed by the presence of a guarding male in it. Therefore, the 'starting date', when males first began to occupy a hole, was only exactly known when a male took over a deserted natural hole which was still being observed.

The 'occupation data' obtained throughout the season, at the various marked natural nest sites, is found in **Appendix I.a**. Males that were only seen inside a nest once or twice, have been excluded from the data set. A total of 17 territorial *B.incognitus* males was observed, at 14 marked natural holes during the whole research period.

Figure I.1 is a cumulative frequency graph of the number of males that stopped occupying their nest during the course of time. Day number has been set out on the x-axis, starting with April 22 being day 0.

We see that 4 (23.5 %), of the total of 17, territorial males have deserted their hole by July 1 (day 70). During July, 10 more males leave their shelter. All 17 males, which were being observed during the research period, have deserted their hole by August 8 (day 108).

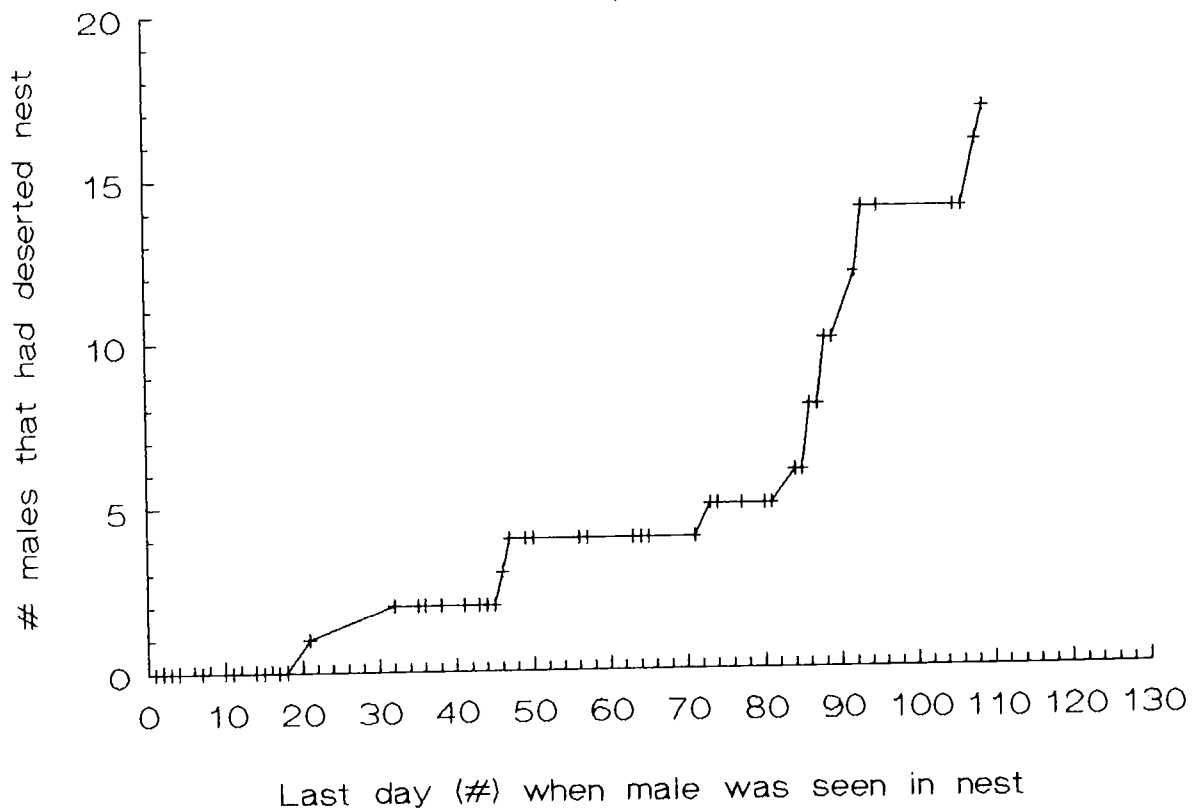


Figure I.1. Cumulative frequency of the number of territorial males which stopped occupying a natural hole, during the course of time.

To get an idea of the length of time that *B.incognitus* males occupy a natural hole, we will assume that the males kept guarding their hole in between successive observations. The males were not marked in any way, but they could be recognized by their body size and colouration, as well as by orbital tentacles, so that the above assumption could be made. In doing so, 'guarding time' was calculated for each of the 17 males observed. This was done by counting the number of days from the first day up to and including the last day that a male was seen to occupy its nest. This guarding time is, of course, an estimate of the numbers of days that males truly occupied their hole, because observations were not made daily and not all males were spotted on their first 'guarding day'. The corresponding data is tabulated in **Appendix I.b**.

For each territorial male, the number of times it was actually seen to occupy its natural hole has been set against the estimated guarding time (in days) in **Figure I.2**. Thus, natural holes that were seen to be occupied daily, have their data point situated on the line $Y=X$. Only one male was observed daily, throughout its short (3 day) guarding period. The remaining data points are situated under the line $Y=X$, as males had not been observed daily.

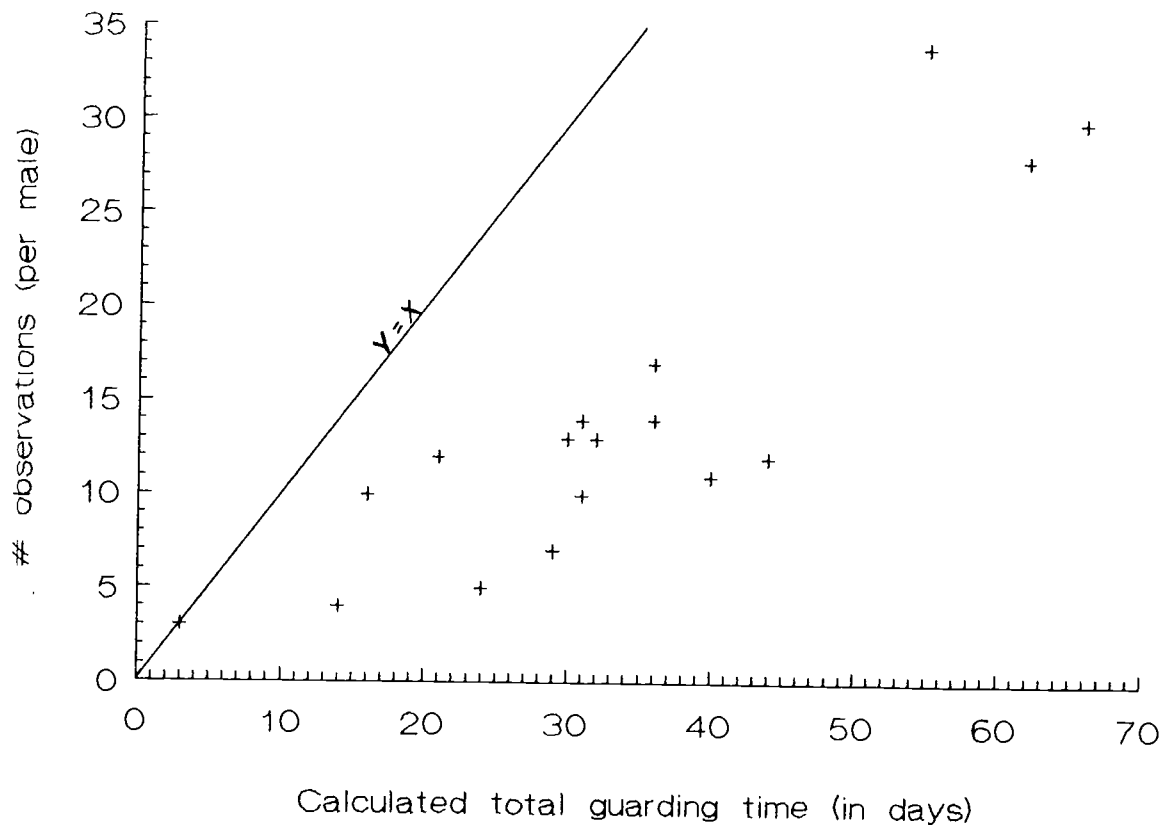


Figure I.2. The number of times territorial males were seen to occupy their natural hole, set against estimated number of 'guarding days'.

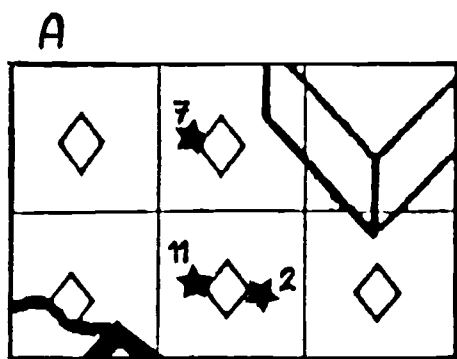
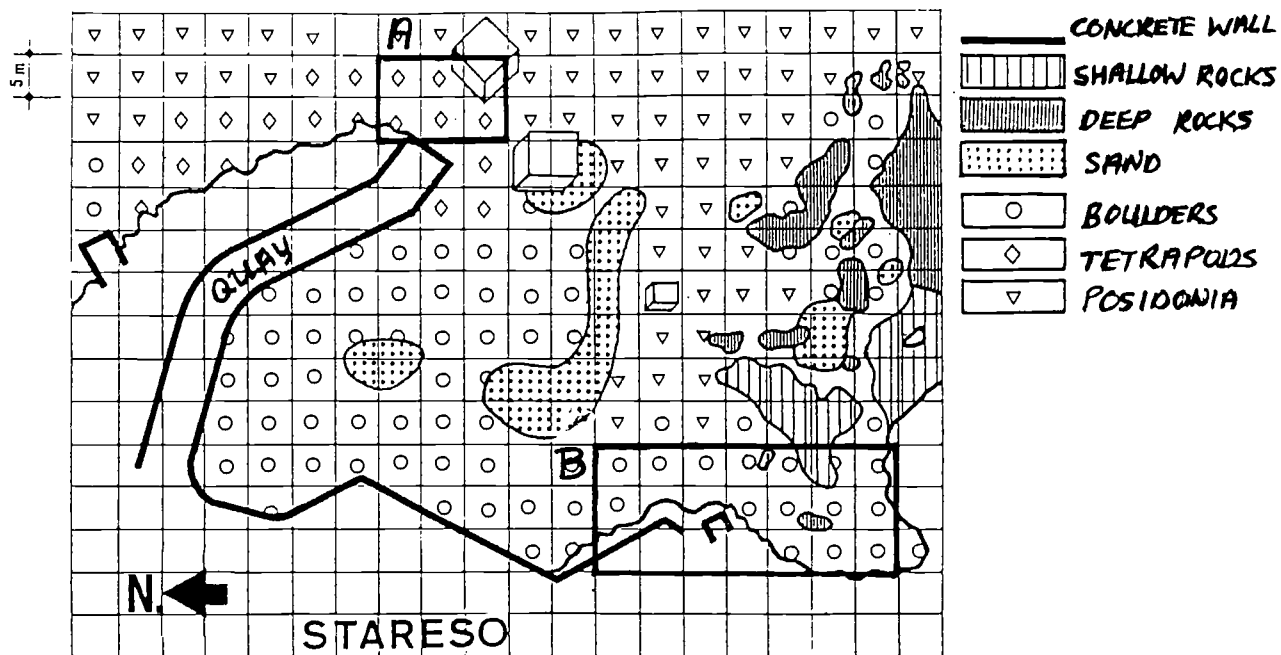
From the graph, we see that only 3 (17.6 %) males have an estimated guarding time of 55 days or more. The remaining 14 (82.4 %) males occupied their nest for less than 45 days, of which 9 (52.9 % of the total) males have an estimated guarding time between 25 and 45 days.

I.B. ECOLOGICAL ASPECTS OF NATURAL HOLES

Several parameters were measured on and around the 14 marked natural holes, which were occupied by *B.incognitus* during the season; the resulting data (including averages and standard deviations) is tabulated in **Appendix I.c.**

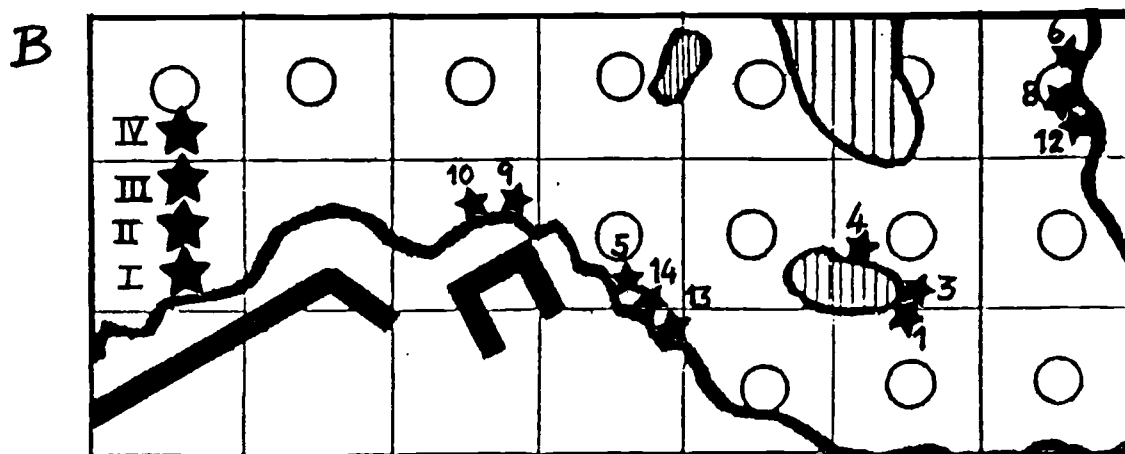
To get a better view of the study area, a map has been made to scale: **Figure I.3.** Two areas (A and B) on the map have been enlarged, to show the positions of the blocks, offering the artificial nest sites

Figure I.3. Map to scale, of the study area at Stareso
(from Wernerus, 1989).



Areas A and B are enlarged from the map, showing the positions of:

- marked natural holes ★
- blocks with 2 types of artificial holes. ★



The smallest distance measured between any two neighbouring territorial *B.incognitus* males, each occupying a marked natural hole, was ± 35 cm between nest 1 and 3 (Appendix I.c). Of the 14 marked holes, the 'distance to the nearest conspecific neighbour' varies between 0.35 and 4.8 m, the average being 1.35 m (s.d.= 1.22). The distance between two artificial holes (test-tubes) in the blocks was ± 10 cm.

The most common species of algae (as seen by the eye), which surrounded the natural nest sites of *B.incognitus*, were the following:

<i>Jania rubens</i>	- Rhodophyta
<i>Corralina elongata</i>	- Rhodophyta
<i>Acetabularia mediterranea</i>	- Chlorophyta
<i>Cladophora prolifera</i>	- Chlorophyta
<i>Udotea petiolata</i>	- Chlorophyta
<i>Padina pavonia</i>	- Phaeophyta
<i>Halopteris filicina</i>	- Phaeophyta

Granite was the common, natural substrate found in the area all along the coastline. Crevices and holes in granite rocks, boulders, and walls served as natural nest sites for several *Blennius* species. The concrete quay wall and the concrete 'tetrapods', weights protecting the base of the wall, also contained suitable nest sites used by several male blennies. Two of the 14 marked natural holes, occupied by *B.incognitus*, were situated in a concrete tetrapod.

Two species of sponge also served as a nest site for *B.incognitus* males. Five oscula of the widespread sponge *Ircinia muscarum* formed shelters for *B.incognitus* and one marked nest site was situated in *Spongia officinalis*. Usually, the natural holes had one large opening (entrance) but in 2 cases a 'tunnel' through a sponge was used as the spawning site, the nest thus having 2 wide openings.

The depth (in metres) under the water surface was measured for each the 14 marked holes shown on the map. Figure I.4 is a frequency distribution graph of measured depth.

We see that 10 (71.4 %) of the 14 holes are situated between depths of 0.5 and 1.0 m. None of the 14 marked holes, which were observed during the season, are situated above 0.5 or below 4.8 m.

In general, the deeper the natural holes were situated, the less exposed they were to waves. Nest sites of *B.incognitus* were not found in places highly exposed to incoming waves. Also, shelters were not found in dark places, such as caves, or underneath overhanging rocks, but in fairly sun-lit places.

Figure I.5 shows the orientation of 12 of the 14 natural holes. The compass directions are expressed as bearings (degrees).

From the figure, we see that most (7 = 58.3 %) hole entrances face \pm South-West, having bearings within the range of 180 to 270°. The remaining 5 holes are orientated in different directions; the average orientation is 196.7° (s.d.= 82.3).

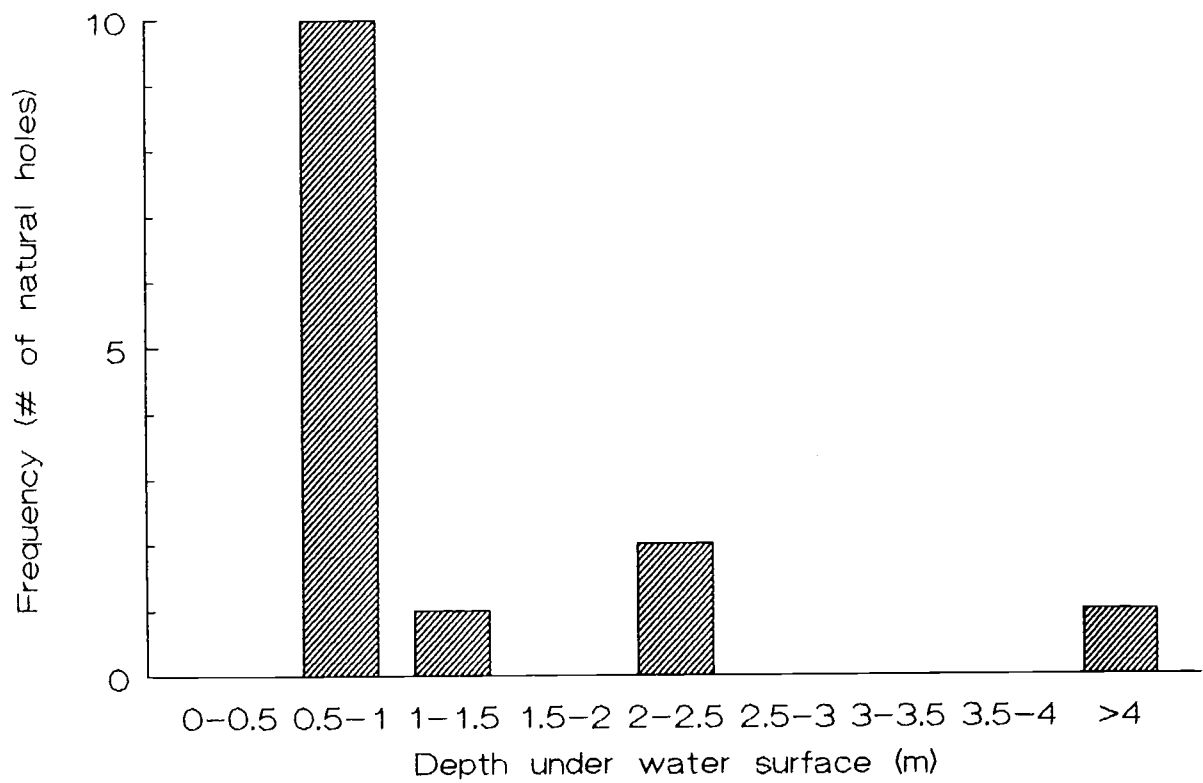


Figure I.4. Frequency distribution of the depth (m) under the water surface, of 14 natural holes.

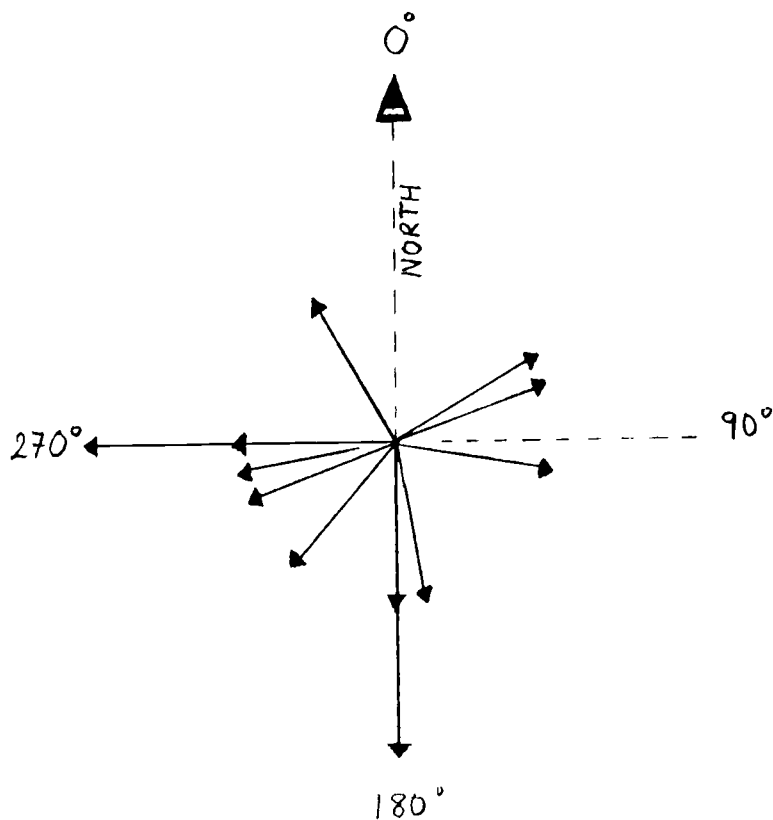


Figure I.5.

*Orientation of 12 natural holes of *B. incognitus*, expressed as bearings (degrees).*

*—→
represents
1 hole.*

In Figure I.6, maximum hole entrance diameter has been set against minimum entrance diameter, for the 14 natural holes. The line $Y=X$ has been drawn in the graph, representing holes with a circular entrance. Also included in the graph are the diameters of the small and large sized test-tubes that were offered as artificial nest sites.

Minimum entrance diameter is seen to vary between 0.73 and 1.68 cm, the mean being 1.13 cm (s.d. = 0.321). Maximum entrance diameter shows a wider range, from 0.73 to 4.27 cm, the mean being 1.56 cm (s.d. = 0.997). Most (10 = 71.4 %) holes have a minimum diameter between 0.7 and 1.2 cm, whilst 11 (78.6 %) have a maximum diameter between 0.7 and 1.8 cm. The diameter sizes of the artificial holes (small tubes measuring 0.85 cm across, large tubes 1.00 cm), compare with the entrance sizes of 9 (64.3 %) marked natural holes. From the graph, we see that 10 (71.4 %) natural holes have entrances which are almost round. Two natural holes have a maximum diameter larger than 3.0 cm; thus clefts (grooves) in the substrate are also used by *B. incognitus*. The regression coefficient for this graph is +0.47. This shows a fair correlation between minimum and maximum hole entrance diameter.

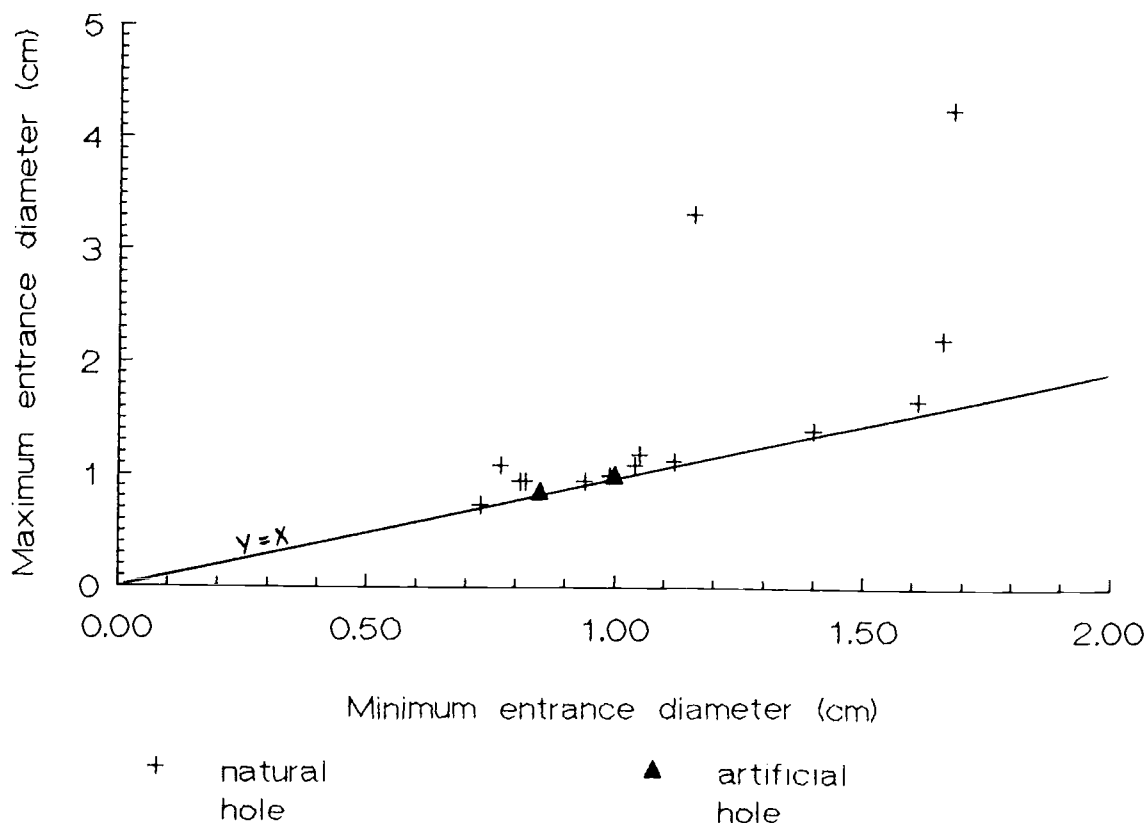


Figure I.6. Maximum hole entrance diameter (cm) against minimum hole entrance diameter (cm), of 14 natural holes and 2 artificial holes.

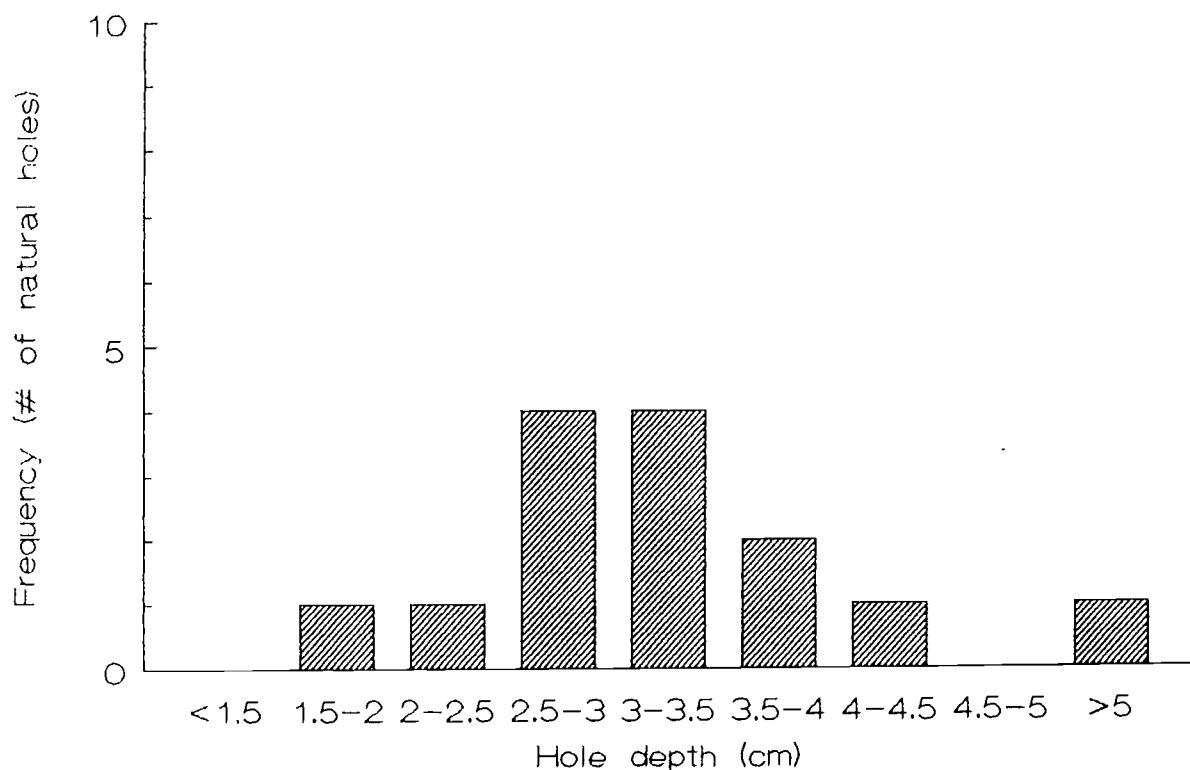


Figure I.7. Frequency distribution of greatest hole depth (cm), for the 14 natural holes of *B. incognitus*.

Figure I.7 is a frequency distribution graph of greatest hole depth, measured for the 14 marked nest sites. Hole depth varies between 1.54 and 5.87 cm, the average hole depth being 32.4 cm (s.d.= 9.70). Most (8 = 57.1 %) of the marked holes have depths between 2.5 and 3.5 cm. Both types of artificial holes have larger hole depths than any of the natural shelters measured; a small tube having a hole depth of 7.9 cm, a large tube having a hole depth of 9.8 cm.

Hole depth has been set against minimum entrance diameter in Figure I.8. Data on the artificial holes has not been included in the graph.

The regression coefficient for this graph is +0.07, showing a negligible correlation between hole depth and minimum entrance diameter.

Hole volume was measured, using glycerol as the testing liquid for 12 holes and vegetable oil in the remaining two cases. Figure I.9 is a frequency distribution graph of hole volume for the 14 marked natural holes.

From the graph, we see that hole volume varies between 2 and 20 ml, although most (8 = 57.1 %) holes have volumes between 2 and 4 ml. The average hole volume is 6.25 ml (s.d.= 5.35).

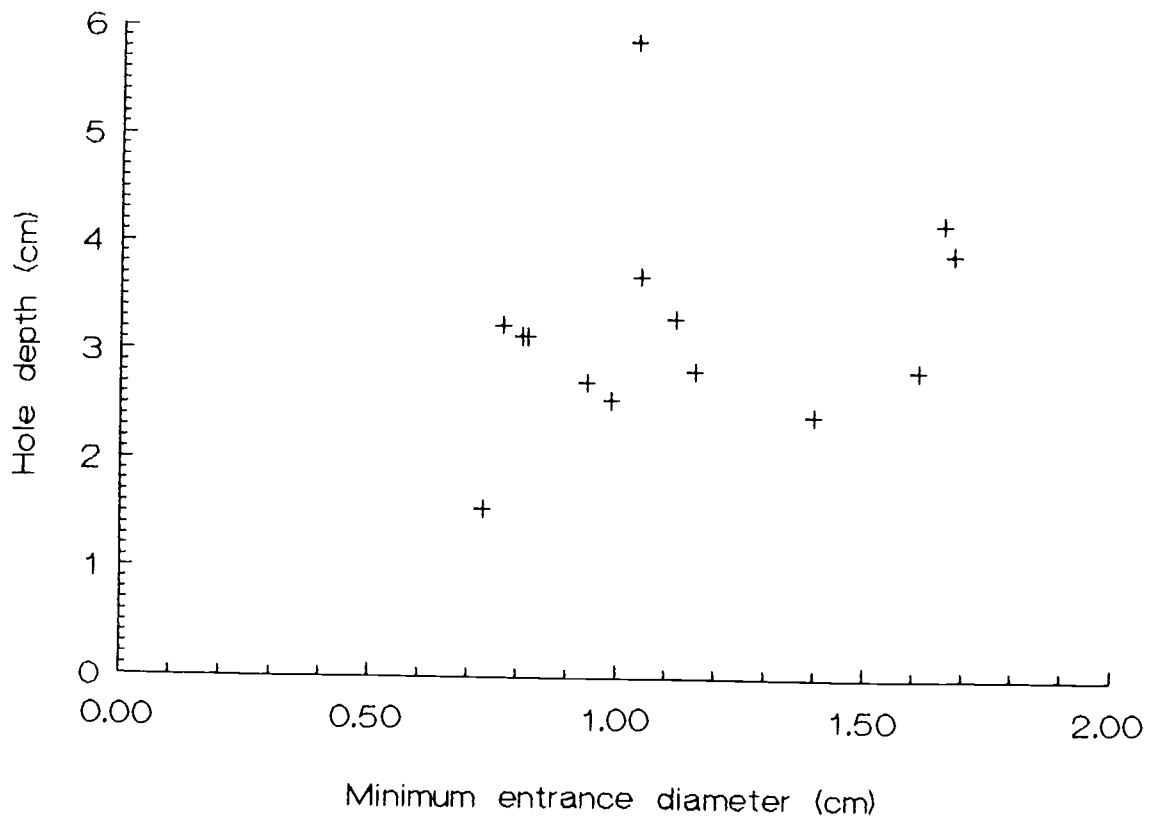


Figure I.8. Hole depth (cm) set against minimum entrance diameter (cm), for 14 natural holes.

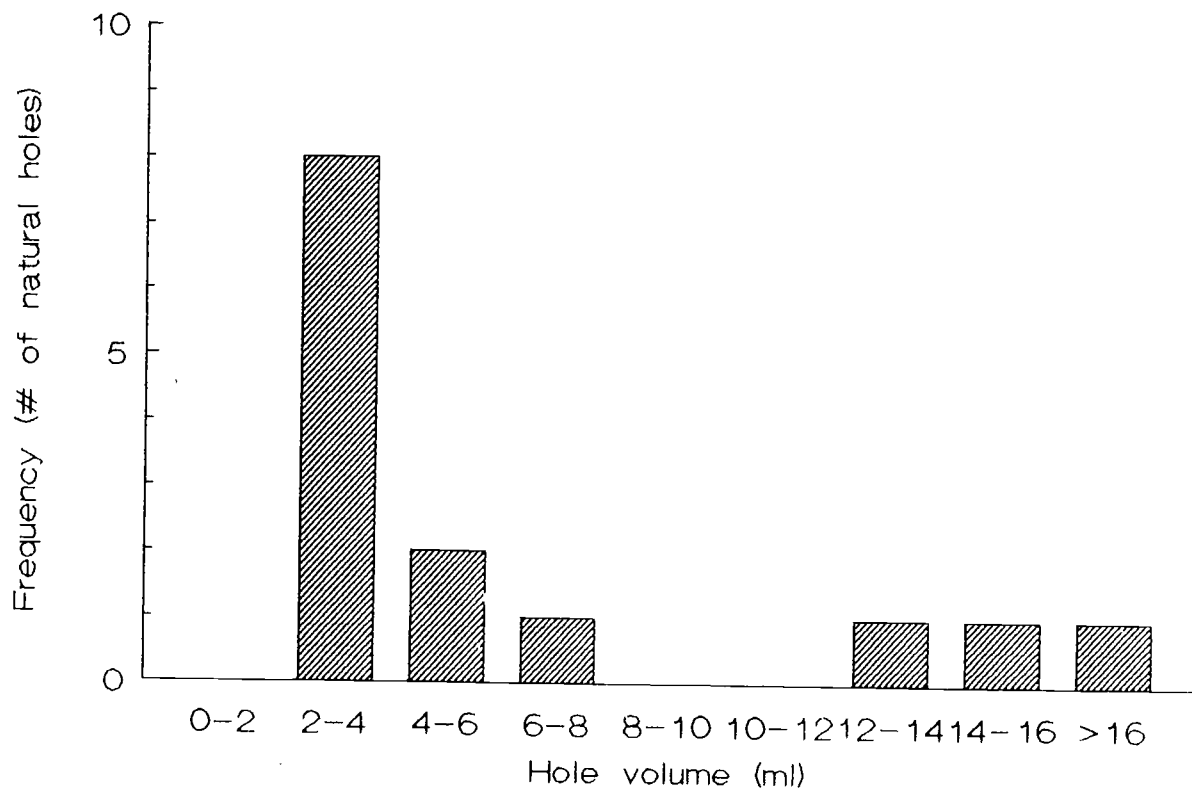


Figure I.9. Frequency distribution of hole volume (ml), of the 14 marked natural holes.

In Figure I.10, hole volume has been set against minimum entrance diameter. Test-tube measurements have been included in the graph.

The regression coefficient is +0.43, showing a fair positive correlation between the two parameters. Compared to the natural nests, both types of artificial holes have relatively large volumes. The volume of a small sized tube (5.0 ml) coincides more closely with volumes of natural holes than that of a large sized test-tube (9.0 ml).

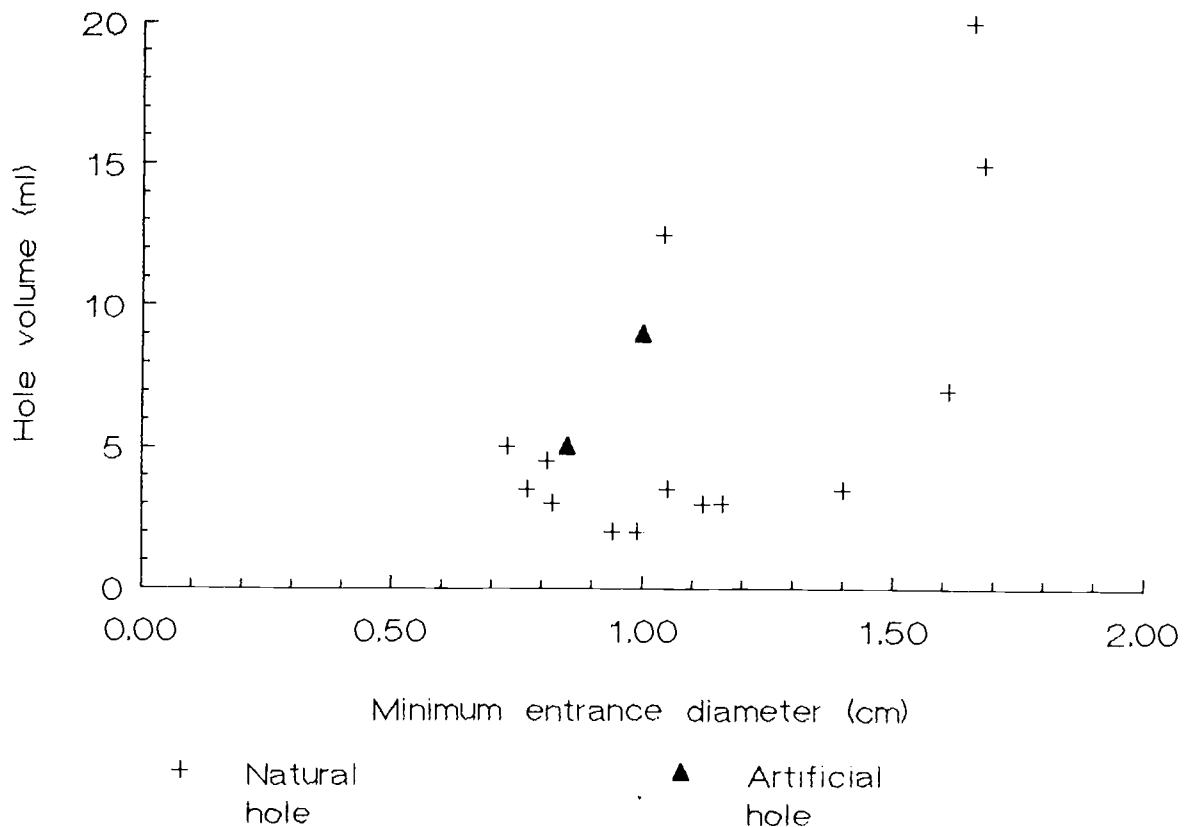


Figure I.10. Hole volume (ml) set against minimum entrance diameter (cm), for 14 natural holes and 2 types of artificial hole occupied by *B. incognitus*.

In Figure I.11, hole volume has been plotted against hole depth, for the 14 marked natural nest sites. Here, we have a regression coefficient of +0.39, showing a reasonable positive correlation between hole volume and hole depth for the marked natural holes. Both sizes of artificial holes which were used, are deeper than any of the natural shelters (data on the artificial nest sites is excluded from the graph).

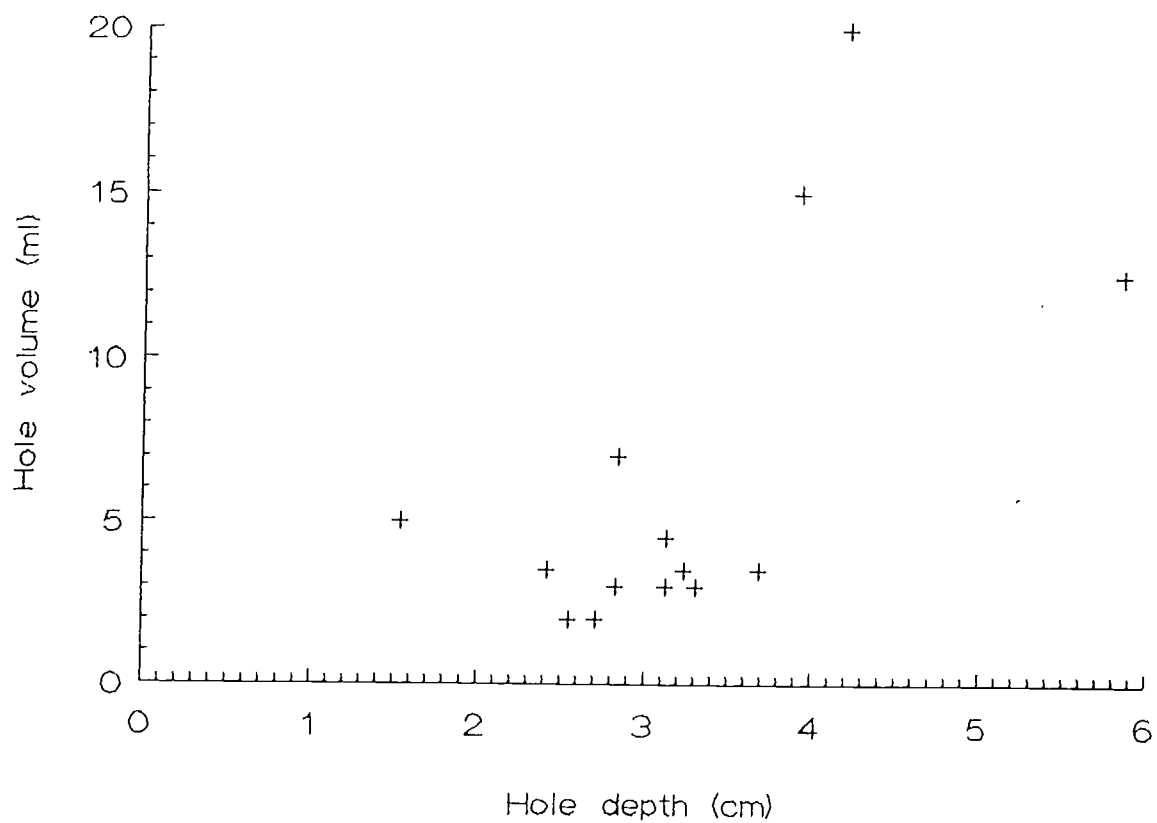


Figure I.11. Hole volume (ml) set against hole depth (cm), for 14 marked natural nest sites of *B. incognitus*.

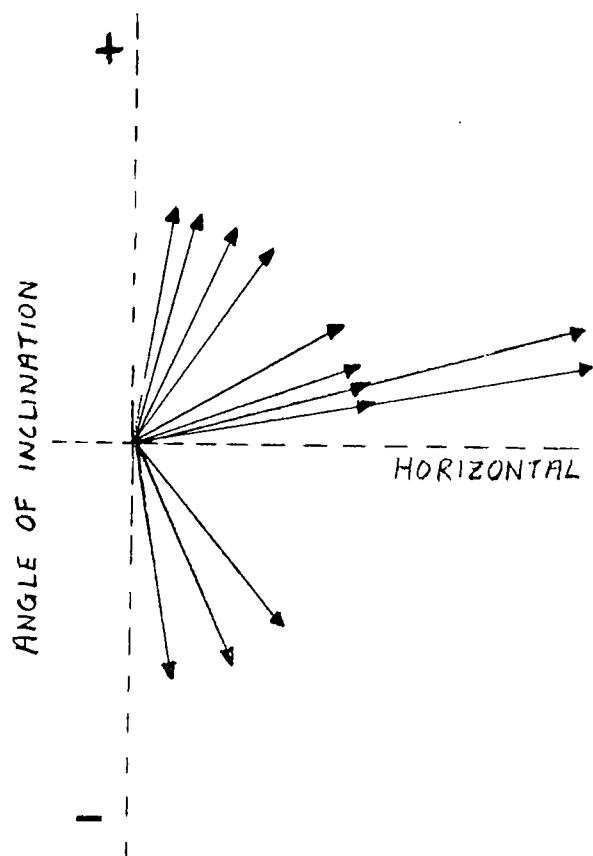


Figure I.12.

Angles of inclination (in degrees), from the horizontal, for the 14 marked natural holes.



represents 1 hole

Figure I.12 shows the angle of inclination measured from the horizontal, for all of the marked natural holes. Positive angles correspond with holes that are 'sloped upwards' (the hole end lying deeper than the hole entrance), whilst negative angles correspond with holes sloping downwards. The angle of inclination varies between $+80^\circ$ and -80° , the average angle being $+16.1^\circ$ (s.d.= 48.4). Of the 14 marked natural holes, 3 (21.4 %) slope downwards, having a negative angle of inclination as measured from the horizontal. The remaining 11 (79.6 %) holes have an upward slope. The artificial holes were placed approximately horizontal, between 0 and $+10^\circ$ incline.

* RESULTS *

----- II. ARTIFICIAL HOLES -----

SUMMARISED RESULTS -----

Only *B.incognitus*, *B.sphinx* and rarely *B.zvonimiri* are seen to use an artificial hole. The holes are occupied by territorial male blennies throughout the research period (May to August); highest levels of occupation occur between mid May and the end of July.

- *B.sphinx* is the main occupant of the test-tubes during May; numbers remain fairly stable between mid May and end June.
- *B.incognitus* starts to occupy tubes in May; numbers increase up until mid June. During June and July, *B.incognitus* is the most common occupant of the 28 offered nest sites.
- After mid July, both *B.incognitus* and *B.sphinx* are found in decreasing numbers; few tubes are occupied by end August.
- *B.zvonimiri* occupies a few tubes, for a short period of time in May.

Block II, at 0.9 m, is occupied most during the whole research period (In 75.9 % of the total number of observations made at this block, a tube is found to be occupied), followed by block I (at 0.4 m) and III (at 1.6 m) having 'overall occupation' values of 64.6 and 35.4 % respectively. Block IV, at 2.1 m, is never seen to be occupied by territorial male blennies.

- *B.sphinx* is found at 0.4 and 0.9 m; it is most frequent at the former depth.
- *B.incognitus* occupies test-tubes at 0.4, 0.9 and 1.6 m, it is least frequent at the shallowest depth.
- *B.zvonimiri* is only seen (rarely) at 0.9 m.

Only blocks I and II are fully occupied over a period of time during June and July. The departure of males is first seen at block III, followed later on by blennies at 0.4 and 0.9 m.

Both sized tubes are occupied at similar rates during the first 10 days that they are offered. Overall, however, more large than small sized tubes are occupied by territorial male blennies. The two tube sizes are occupied by different *Blennius* species in different ratios.

- Throughout the season, *B.sphinx* seems to prefer the large sized tubes.
- Overall, small sized tubes are occupied more often by *B.incognitus* than by *B.sphinx*; these 2 species occupy the large and small sized test-tubes in different ratios, depending on the depth under the water surface.
- *B.zvonimiri* plays too small a role for preferences in tube size to be seen.

Differences in overall occupation of small and large sized tubes by the different *Blennius* species, are significant (Chi-square, $p > 5\%$) for blocks I and III. This is not the case for block II, where the different species occupy similar numbers of small and large artificial holes, as seen over all observations made during the season.

The different species occupy the test-tubes placed at the four different depths in different ratios. Differences in overall occupation are significant (Chi-square, $p > 5\%$) for the small test-tubes, and for the large sized artificial holes.

Thus:

- *B.sphinx* is mostly seen to occupy large sized test-tubes, especially at 0.4 m.
- *B.incognitus* occupies both small and large sized tubes.
- At 1.6 m, *B.sphinx* males being absent at block III, *B.incognitus* is found to occupy more large sized test-tubes than small sized ones. At 0.9 m, *B.incognitus* and *B.sphinx* occupy the different test-tube sizes in similar numbers. At 0.4 m, *B.sphinx* is the more frequent occupant; here, *B.incognitus* is never seen to occupy a large sized tube.

The water temperature rose from 14°C during April, to 26°C by August 17 (day 117). Most *B.sphinx* males started occupying a test-tube during May, when temperatures varied between 14 and 16°C. *B.incognitus* also started to occupy test-tubes during May, but most males arrived during the beginning of June, when temperatures had passed 16°C.

All of the 14 natural holes, occupied of *B.incognitus*, are deserted by August 8 (day 108), whilst 2 test-tubes are still occupied by territorial males on August 28 (day 128)

II.A. OCCUPATION OF ARTIFICIAL HOLES

The results on the occupation of the artificial holes, gained between May 6 (day 14) and August 28 (day 128), are shown per 'test-tube observation' in Appendix II.a. Line graphs that follow, show 'levels of occupation' (expressed in percentages) set against time.

At the end of August, the 'occupation data' for each test-tube was summarised over the whole research period. Thus, per tube, frequencies were obtained for the different *Blennius* species which had been encountered in a tube during the season, as well as the number of times a tube was found to be unoccupied. This summarised data, or 'overall occupation' data, is given in Appendix II.b (compensated data for block I is included). In figures on overall occupation, data is expressed in percentages.

The overall occupation of all (28) offered artificial holes (irrespective of tube size or depth) is shown in Figure II.1. The data is expressed in percentages, over the total number of observations made during the research period; one observation being equal to the checking of one tube.

We see that a test-tube is unoccupied in 56.0 % of the total number of observations (1276 compensated as 1344) made during the research period. *B.incognitus* is the most frequent 'overall occupant species' (22.0 %), followed closely by *B.sphinx* (21.2 %). *B.zvonimiri* is also seen, but in only 0.8 % of the observations. No other species of fish is seen to use the artificial holes during the daytime observations.

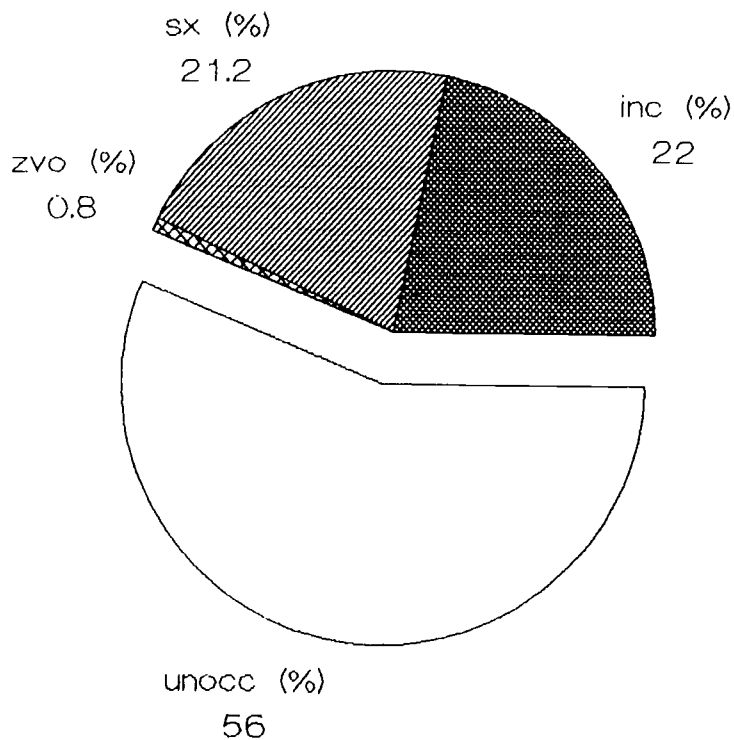


Figure II.1. Overall occupation (percentages) of all (28) artificial holes, calculated at the end of the season.

In order to visualize the occupation of the artificial holes during the course of time, the number of tubes found to be occupied ('occupation level') per day, could be expressed as a percentage over the total number of test-tubes offered. This was done for each 'daytime observation period', when all (28) artificial holes were checked within half an hour during the day. The data is tabulated in Appendix II.c.

The 'level of occupation' (in percentages), of 28 artificial holes, during the course of time is displayed in Figure II.2. The tubes were first offered on May 6 (day 14 of the research period. The x-axis starts with April 22 (day 0). Only 10 days after the blocks are offered (day 24), $\pm 40\%$ of all the offered tubes is occupied by a territorial male blenny. This level of occupation remains $\pm 40\%$ until the beginning of June (day 40), when more territorial males start to occupy a tube; the occupation level rises to $\pm 65\%$. July (day 70-100) shows a decrease in occupation level from $\pm 65\%$ to $\pm 40\%$. This decrease continues through August and only $\pm 15\%$ of the 28 test-tubes is occupied on the last observation day, August 28 (day 128).

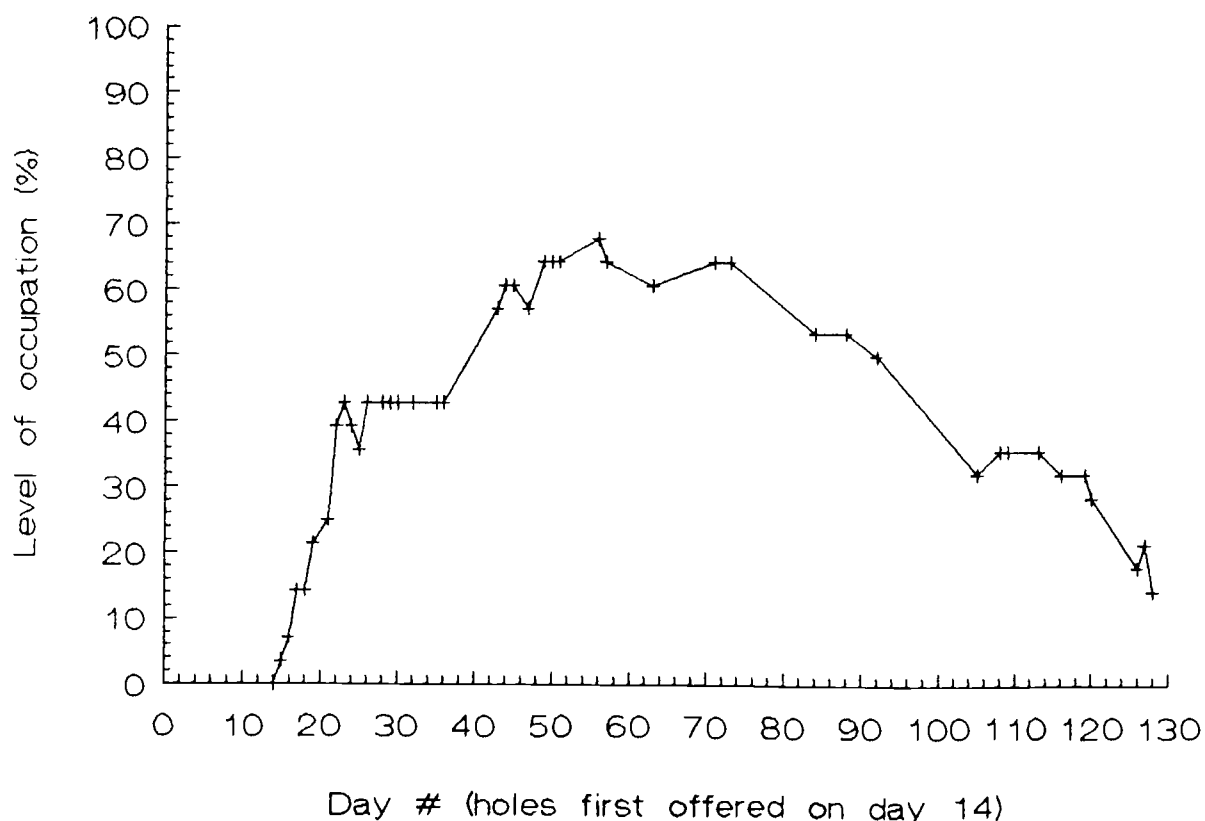


Figure II.2. Level of occupation (in percentages) of all (28) artificial holes, against time.

The occupation of the artificial holes by each of the three occupant species (*B.incognitus*, *B.sphinx* and *B.zvonimiri*) during the course of time, is displayed in Figure II.3. The occupation levels (number of test-tubes seen to be occupied) are given in percentages, calculated over the total number (28) of test-tubes offered.

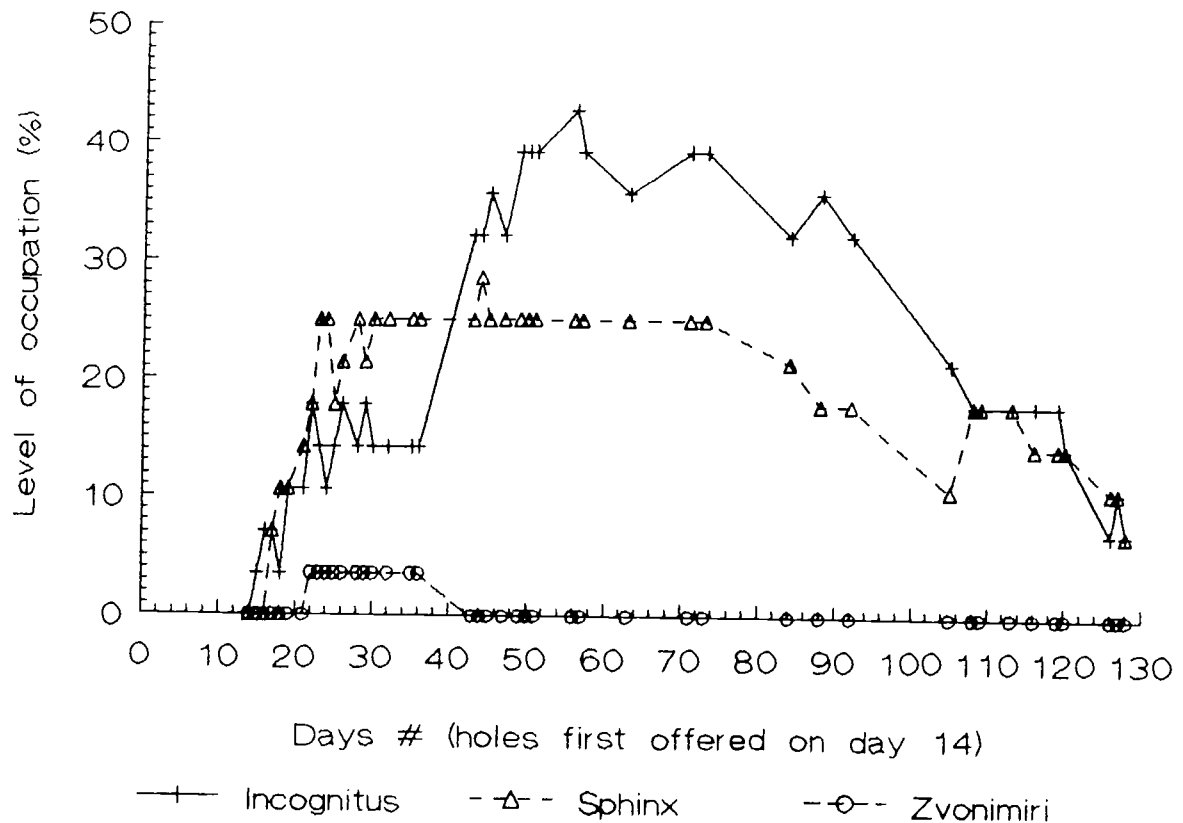


Figure II.3. Level of occupation (in percentages) of the (28) artificial holes by 3 *Blennius* species, against time.

From the graph, we see that similar numbers of *B. incognitus* and *B. sphinx* are present in the tubes, during the first 10 days in which they were offered. During the second half of May (day 23-39), *B. sphinx* occupies $\pm 25\%$ of the 28 tubes, whilst *B. incognitus* occupies $\pm 15\%$. With the coming of June, more *B. incognitus* males choose to inhabit an artificial hole so that, in June (day 40-69), average levels of occupation vary between 35% and 40%. *B. sphinx* keeps $\pm 25\%$ of the tubes occupied; this value decreases during July (day 70-100), to $\pm 15\%$. The number of tubes occupied by *B. incognitus* also decreases during July, down to $\pm 25\%$. During August, both species occupy the 28 artificial holes in similar, low numbers. *B. zvonimiri* is only seen to occupy a few tubes during the second half of May, over a short period of time. Less than 4% of all the test-tubes is ever occupied by this species during the whole research period.

Thus, the different *Blennius* species use the artificial holes as a nest site throughout the research period. Highest levels of occupation are found between mid May and the end of July; *B. sphinx* is the main occupant early on in the season, *B. incognitus* is the major occupant in the latter part of the season.

II.B. ARTIFICIAL HOLES: DEPTH

To get an idea of the occupation of test-tubes (irrespective of size) placed at the different depths, overall occupation percentages have been calculated per block. The data on overall occupation, for each of the four blocks (placed at 0.4, 0.9, 1.6 and 2.1 m), can be found in **Appendix II.b** (compensated data on block I included).

Figure II.4 shows the overall occupation (percentages) of block I, by the different *Blennius* species. Percentages have been calculated from (compensated) frequencies, over the total number of observations (168 compensated as 336) made at 0.4 m (block I).

Overall, *B.sphinx* is the most frequent occupant species of the 4 tubes of block I. This species is encountered in 47.9 % of the (compensated) total number of observations. In 16.7 % of the observations, *B.incognitus* is seen to reside in a tube. In the remaining 35.4 %, a tube is found unoccupied.

Figure II.5 shows the overall occupation (percentages), of block II, which was placed at 0.9 m.

B.incognitus is encountered in 35.7 % of the total number (336) of observations made at block II. This is very similar to the number of times (36.9 %) in which *B.sphinx* is the occupant of a test-tube. In a rare 3.3 % of the cases, *B.zvonimiri* is seen to occupy a tube, situated at 0.9 m. No occupant is found in the remaining 24.1 % of the observations.

Figure II.6 shows the overall occupation (percentages) of block III, placed at 1.6 m under the water surface.

B.incognitus is the only species encountered in the artificial holes at 1.6 m. Territorial males are met in 35.4 % of the total number of observations (336) made at block III. In the remaining 64.6 % of the observations, a tube is found unoccupied.

Figure II.7 shows the overall occupation of block IV, which was situated at 2.1 m under the water surface.

Throughout the whole research period, territorial male blennies, or other species of fish, are not seen to occupy an artificial hole of block IV, at 2.1 m.

The differences in frequencies of overall occupation (by the various *Blennius* species) observed between the four blocks, are shown to be highly significant ($p > 5\%$) using the Chi-square test.

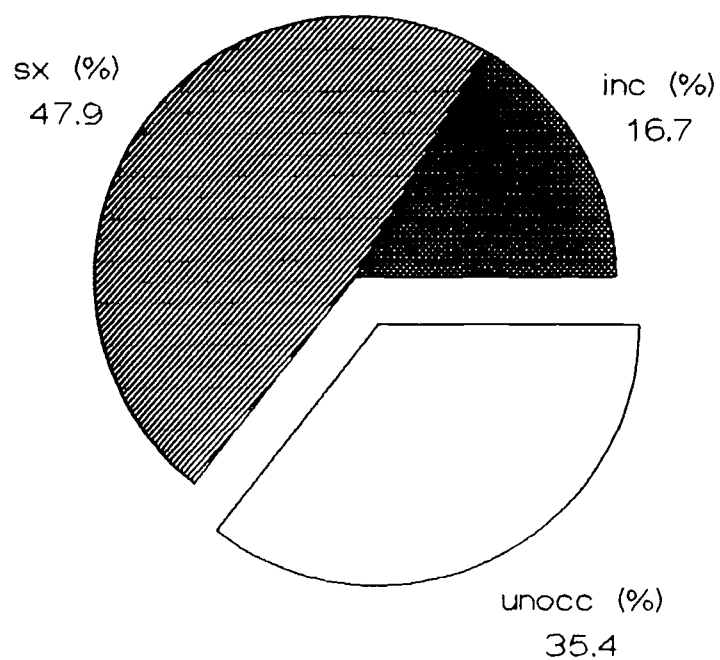


Figure II.4. Overall occupation (in percentages) of the 4 test-tubes at 0.4 m (block I), calculated at the end of the research period; data has been compensated.

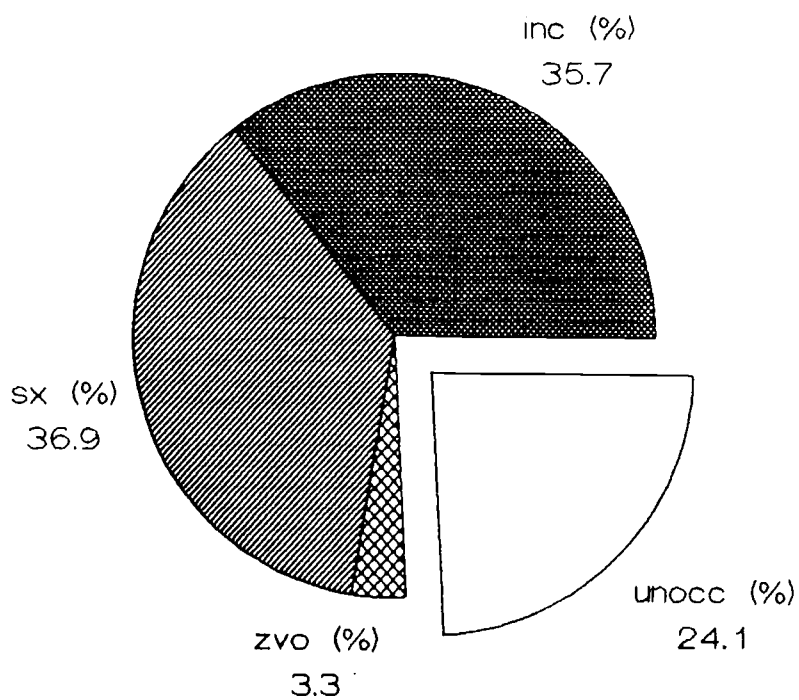


Figure II.5. Overall occupation (in percentages) of the 8 test-tubes at 0.9 m (block II), calculated at the end of the research period.

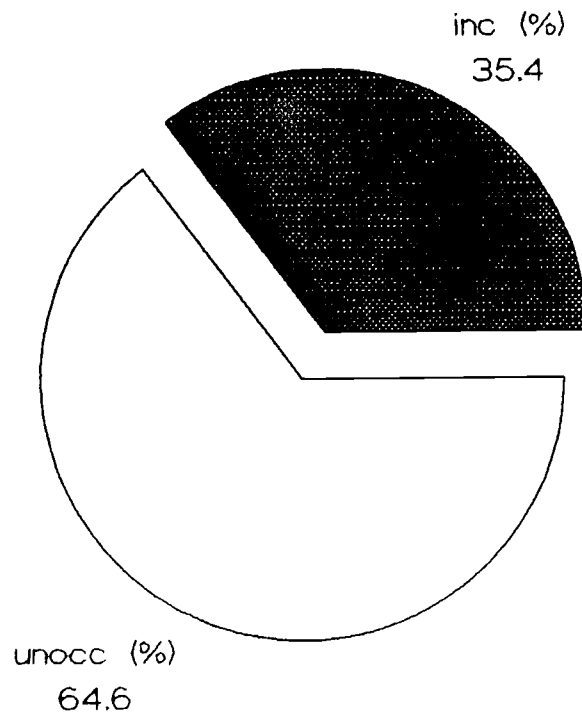


Figure II.6. Overall occupation (in percentages) of the 8 test-tubes at 1.6 m (block III), calculated at the end the research period.

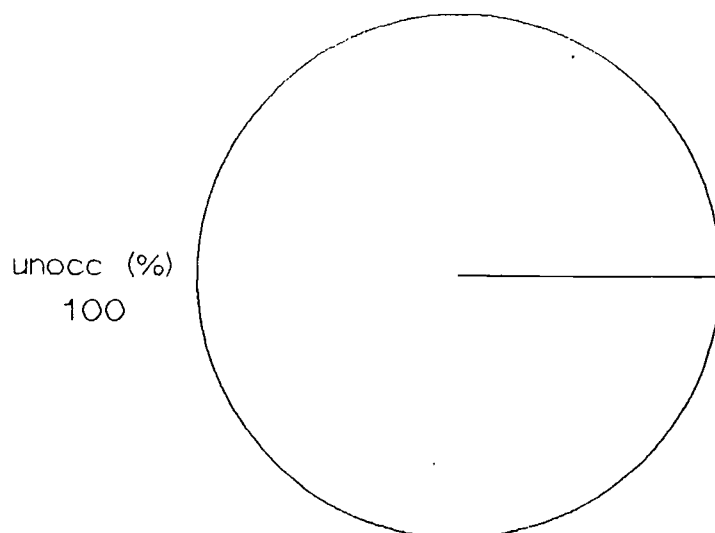


Figure II.7. Overall "occupation" of the 8 test-tubes at 2.1 m (block IV), calculated at the end the research period.

Occupation levels of each block, during the course of time, are displayed in Figure II.8. The levels of occupation are expressed as percentages, calculated over the total number of test-tubes offered per block (i.e. per depth). The data is given in Appendix II.d.

From the graph, we see that both blocks I and II (at 0.4 and 0.9 m respectively) are fully occupied on May 21 (day 29), only 15 days after they are first offered. The 8 test-tubes of block II remain occupied until July 21 (day 90). The four test-tubes in block I are all occupied between day 44 and day 90. During May (up to day 39), 1 ($\pm 12\%$) of the (8) test-tubes of block III is occupied. This number increases in June, to a maximum of 87% (7 tubes) on June 17 (day 56). During July, males of block III leave their artificial hole. The males in blocks I and II desert their tube later on in the season, during the last week of July (day 93-100). No test-tubes at 0.4 m are occupied on the last day, August 28 (day 128). Only $\pm 25\%$ (2 tubes) at 0.9 and at 1.6 m are still occupied at this point in time. The so-called 'occupation' of block IV may be represented by the line $Y=0$.

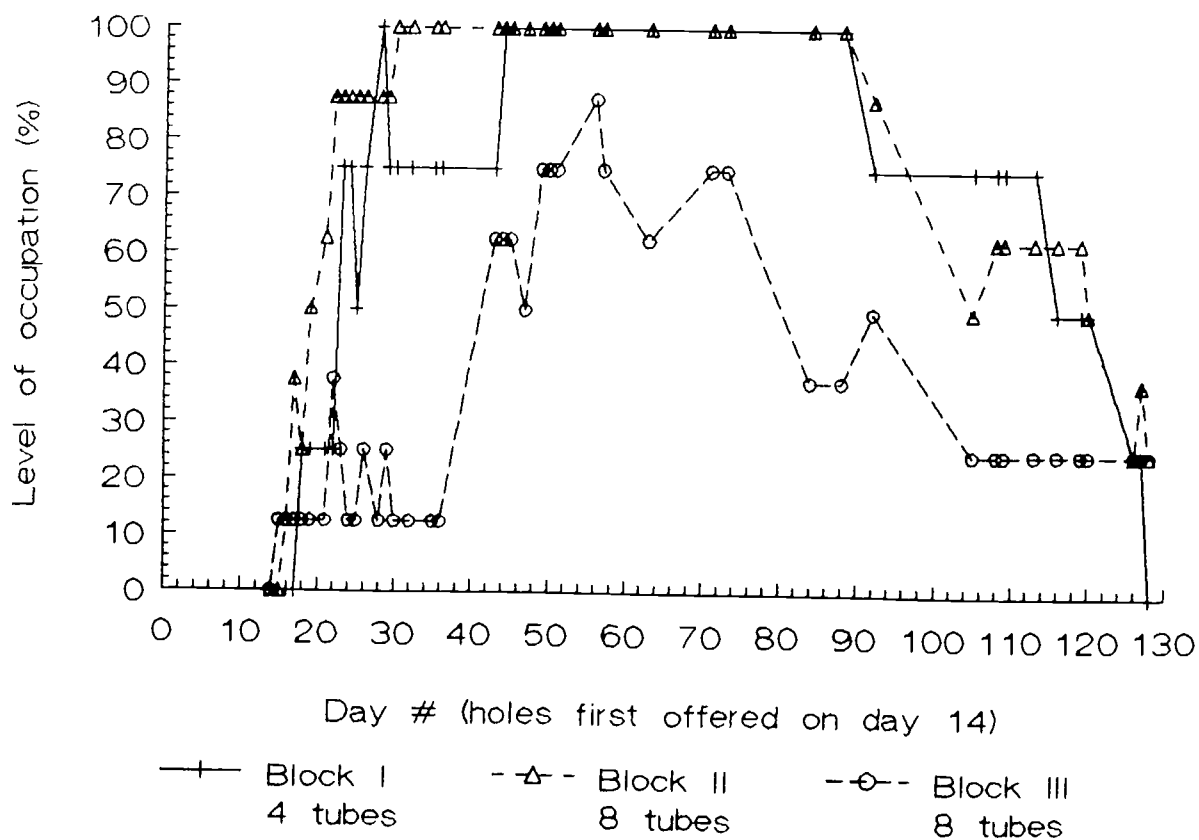


Figure II.8. Level of occupation (in percentages) of blocks I, II and III, against time; the occupation of block IV may be represented by the line $Y=0$.

II.C. ARTIFICIAL HOLES: SIZE

The level of occupation of small and of large sized test-tubes during the course of time, irrespective of depth under the water surface, is shown in Figure II.9. Percentages have been calculated either over the number of small (13) or over the number of large (15) sized tubes.

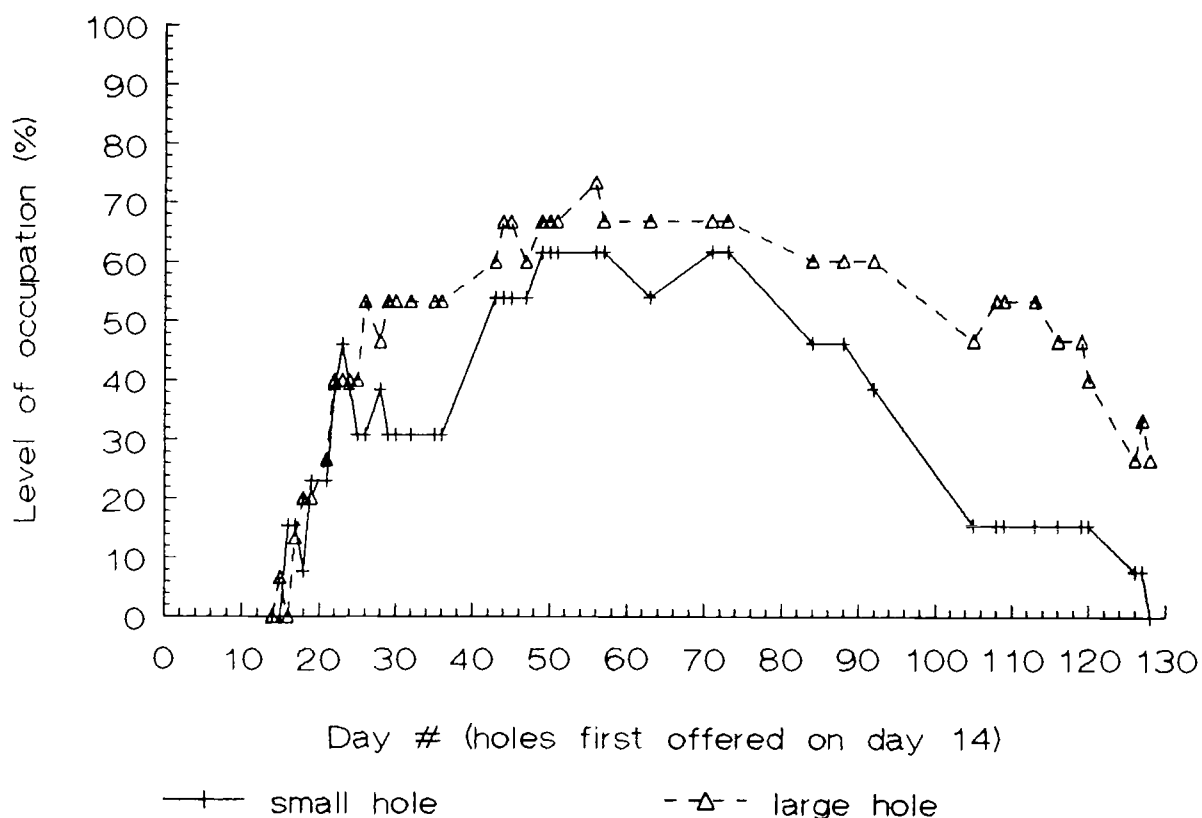


Figure II.9. Level of occupation (in percentages) of 13 small and 15 large sized test-tubes, against time.

During the first 10 days (day 14-24) in which the tubes are offered, both sizes are occupied at similar rates. During the rest of the research period, the large sized test-tubes are occupied more often than the smaller sized ones. The difference between the occupation levels of small and large artificial holes is least during June (day 40-69), varying between 5 and 15 %. During the second half of May, this difference varies between ± 10 and 23 %. During the month of August (day 101-128) the difference is greatest, between ± 10 and 30 %.

Figure II.10 shows the overall occupation (percentages) of the 13 small artificial holes (irrespective of depth under the water surface).

Of the total number of observations (546 compensated as 672) made at small test-tubes, 61.0 % show a small sized tube to be unoccupied. In only 0.5 % of the observations, *B.zvonimiri* is the occupant. *B.sphinx* accounts for 15.0 % of the cases, whilst *B.incognitus* is seen in 23.5 % of the observations.

Figure II.11 shows the overall occupation (percentages) of the 15 large artificial holes (irrespective of depth under the water surface).

Of the total number of observations (630 compensated as 672) made at large sized tubes, 51.0 % show a medium sized tube to be unoccupied. *B.zvonimiri* is seen in 1.2 % of the cases. *B.sphinx* is the occupant in 27.4 % of the observations and *B.incognitus* in 20.4 %.

The difference in the frequency of (overall) occupation by the various *Blennius* species, observed between the small and large sized holes, is shown to be significant ($p > 5\%$), using the Chi-square test.

Figure II.12 shows occupation levels (in percentages) of the 13 small sized test-tubes during the course of time. The corresponding data is given in Appendix II.e.

B.incognitus and *B.sphinx* occupy small test-tubes in similar rates during the month of May. During June and July (day 40-100), *B.incognitus* regularly occupies more than twice the number of small tubes which are being occupied by *B.sphinx*. The former species occupies between 4 and 6 (± 31 and 46 %) small sized tubes during this period, whilst *B.sphinx* occupies between 1 and 3 (± 8 and 23 %) small tubes. After mid August, 1 male (± 8 %) of each species is left, occupying a small tube. Only 1 *B.zvonimiri* male is seen to occupy a small sized tube at the end of May.

Figure II.13 shows occupation levels (in percentages) of the 15 large sized test-tubes during the course of time. The corresponding data is given in Appendix II.f.

Occupation percentages for *B.incognitus* and *B.sphinx* rise steadily during May; the latter species occupies ± 33 % (5 holes) by day 30, the former by day 45. Occupation levels for *B.sphinx* decrease after day 84; those for *B.incognitus* decrease after day 92. If we compare the occupation 'curves' of *B.incognitus* and *B.sphinx*, we see that occupation levels of *B.incognitus* 'lag behind' those of *B.sphinx* by 1-2 weeks. Thus, throughout the research period, *B.incognitus* is seen to occupy similar numbers of large sized test-tubes as *B.sphinx*, but does this 1-2 weeks later in time. During May, only 1 (± 6.5 %) *B.zvonimiri* male is seen.

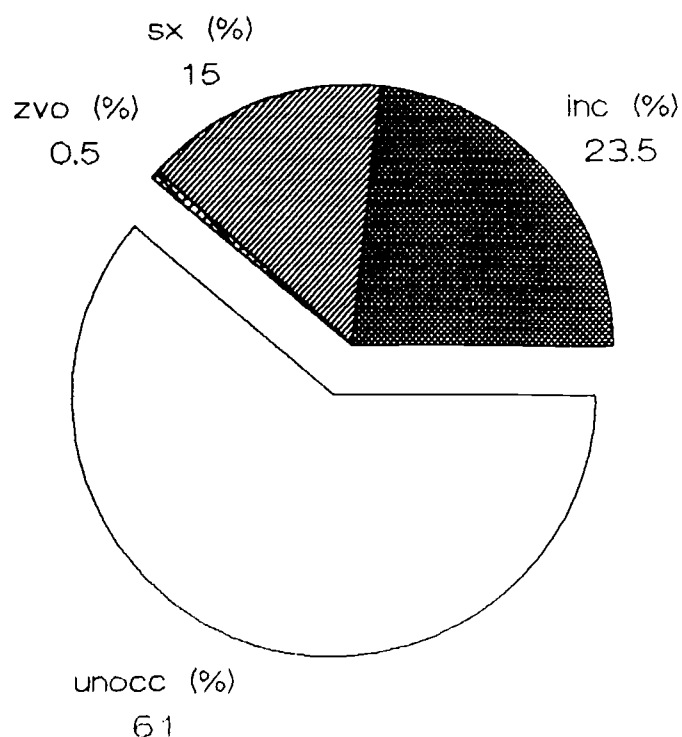


Figure II.10. Overall occupation (in percentages) of the 13 small sized artificial holes, calculated at the end of the research period.

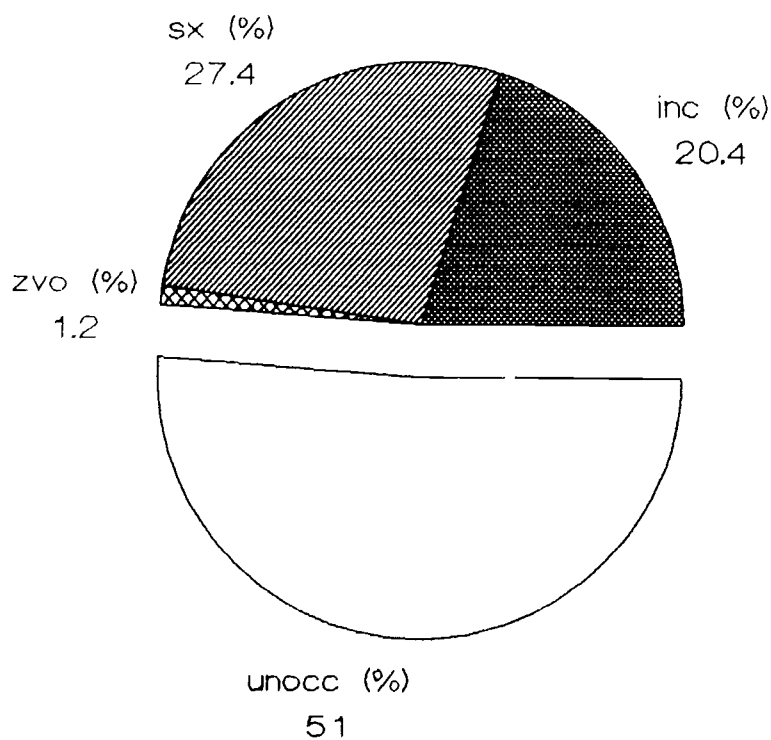


Figure II.11. Overall occupation (in percentages) of the 15 large sized artificial holes, calculated at the end of the research period.

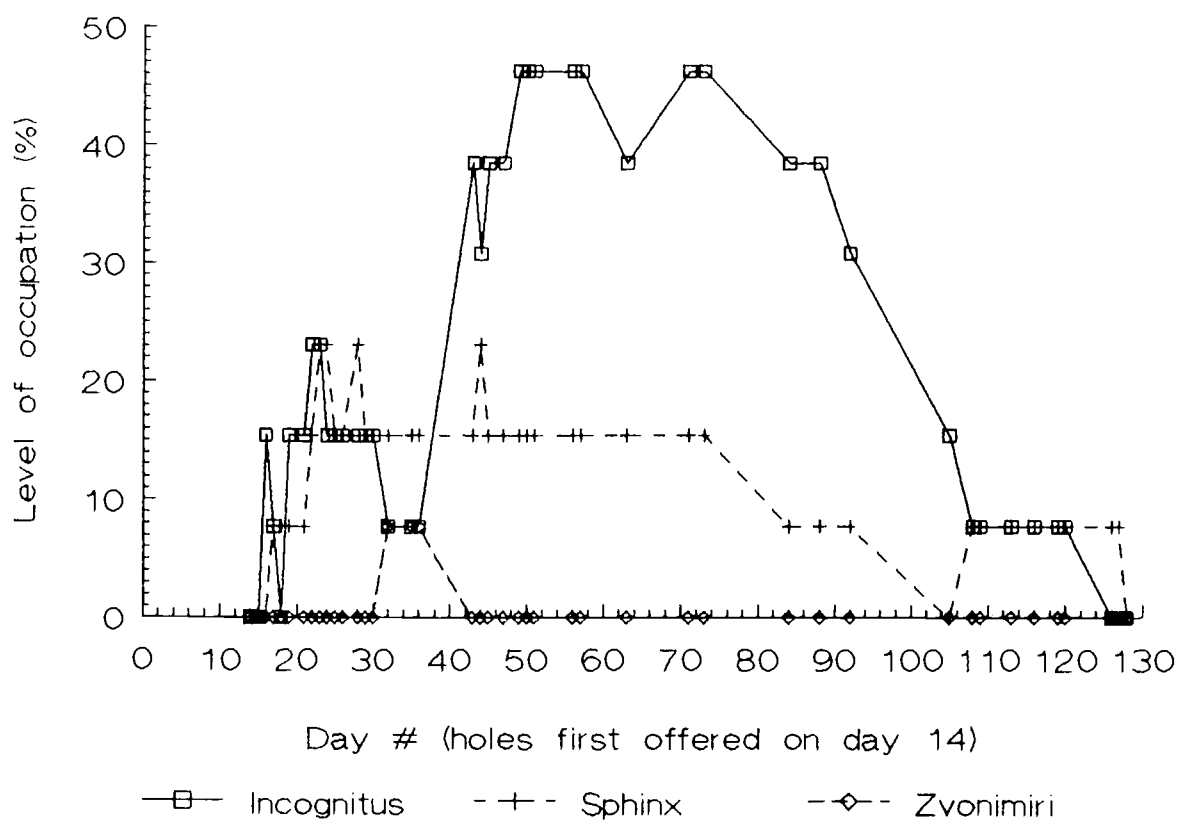


Figure II.12. Level of occupation (in percentages) of the 13 small sized test-tubes, against time.

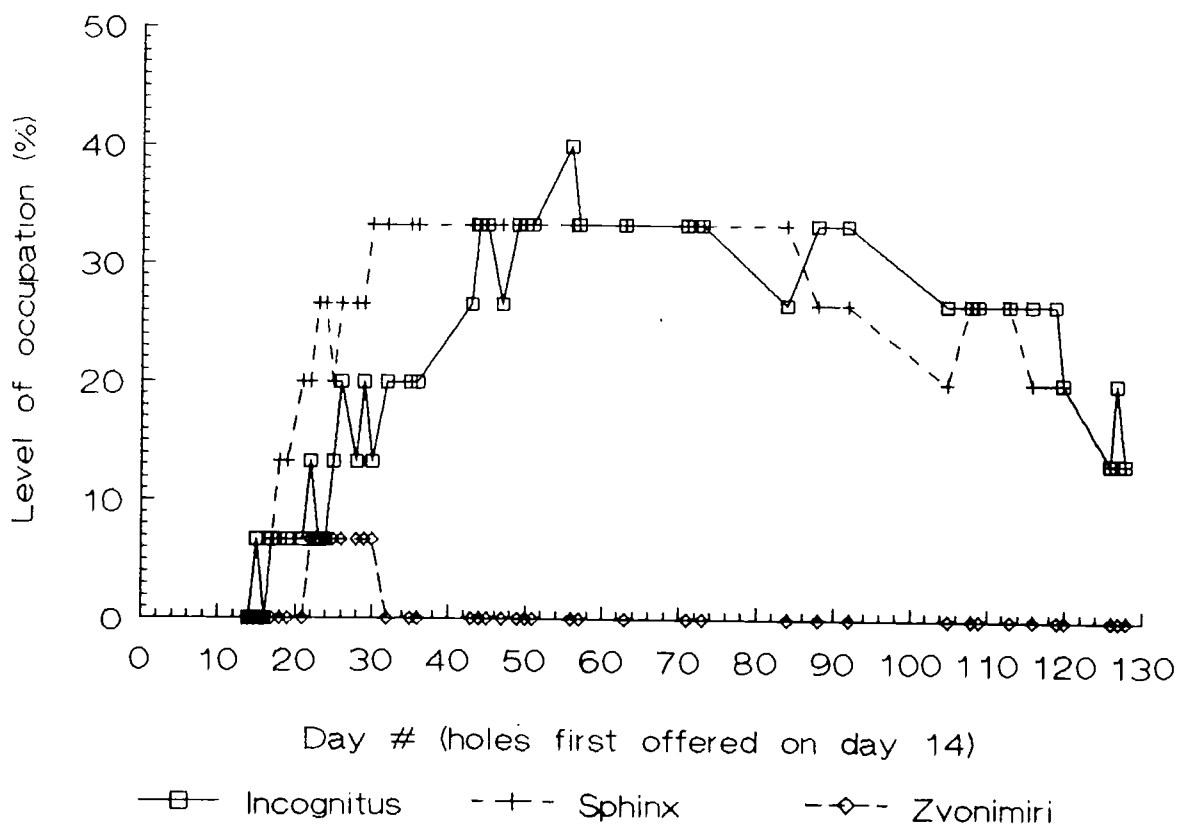


Figure II.13. Level of occupation (in percentages) of the 15 large sized test-tubes, against time.

II.D. ARTIFICIAL HOLES: DEPTH AND SIZE

So far, we have looked at 'overall occupation' and at 'levels of occupation against time'; comparing data of different depths or different tube size. Figure II.14 shows the overall occupation per tube size, per depth.

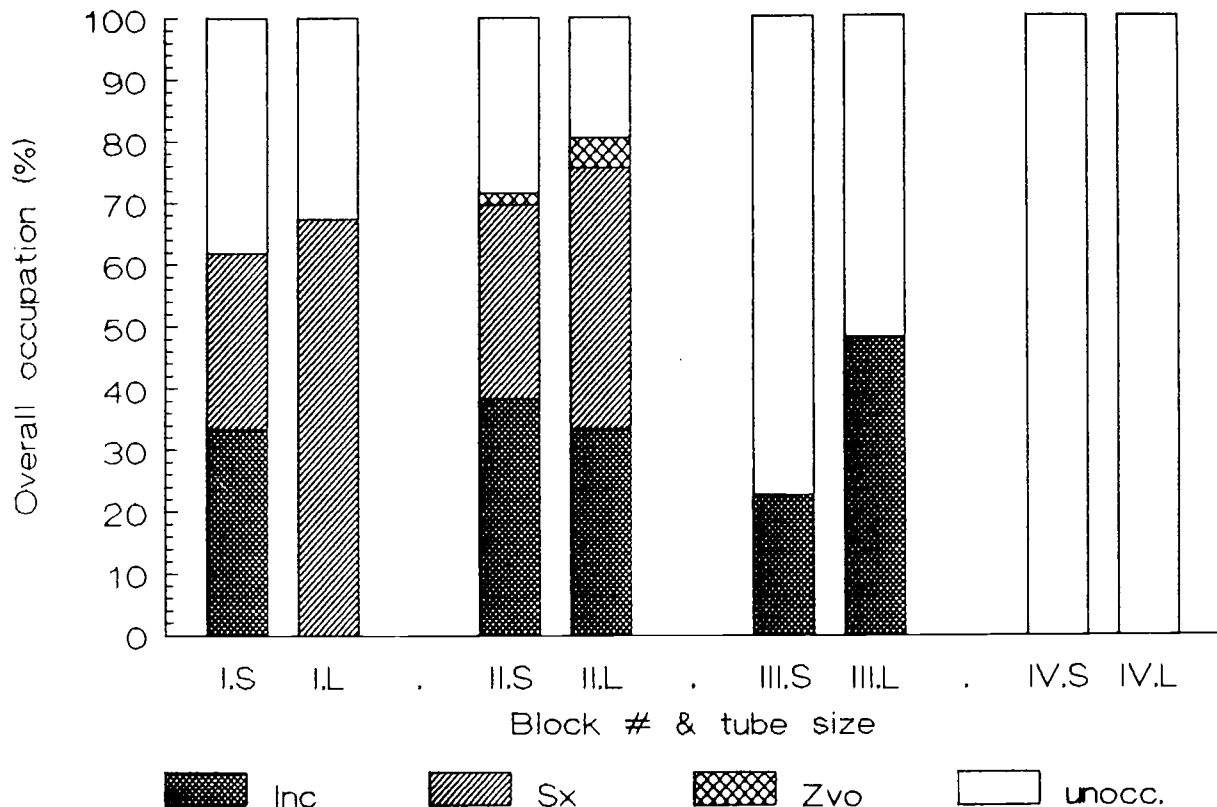


Figure II.14. Overall occupation (in percentages) of small and large sized test-tubes, per depth under the water surface (calculated at the end of the research period).

Block II shows highest overall occupations, for both sized tubes, followed by blocks I and III. *B.sphinx* is found in both small and large sized tubes of blocks I and II, and mostly occupies large sized tubes, at 0.4 m. *B.incognitus* occupies both small and large sized tubes of blocks I, II and III. At 1.6 m (*B.sphinx* males being absent) *B.incognitus* is more frequently found in large sized test-tubes than in small sized ones. At 0.9 m, *B.incognitus* and *B.sphinx* occupy the different test-tube sizes in similar numbers. At 0.4 m, *B.sphinx* is the more frequent occupant; here, *B.incognitus* is never seen to occupy a large sized test-tube. *B.zvonimiri* is rarely seen at 0.9 m, in small and large sized tubes.

The Chi-square test was used, to test the differences in overall occupation within depth and within test-tube size for significance.

WITHIN DEPTH:

- The overall occupation of small and large sized test-tubes is significantly ($p > 5\%$) different at 0.4 m (BLOCK I).
- The overall occupation of small and large sized test-tubes is NOT significantly ($p > 5\%$) different at 0.9 m (BLOCK II).

WITHIN SIZE:

- The differences in the overall occupation of SMALL sized test-tubes between the four depths is significant ($p > 5\%$).
- The differences in the overall occupation of LARGE sized test-tubes between the four depths is significant ($p > 5\%$).

II.E. WATER TEMPERATURE

The water temperature, measured daily between April 22 (day 0) and August 17 (day 117) is displayed in Figure II.15. The data is also tabulated in Appendix II.g.

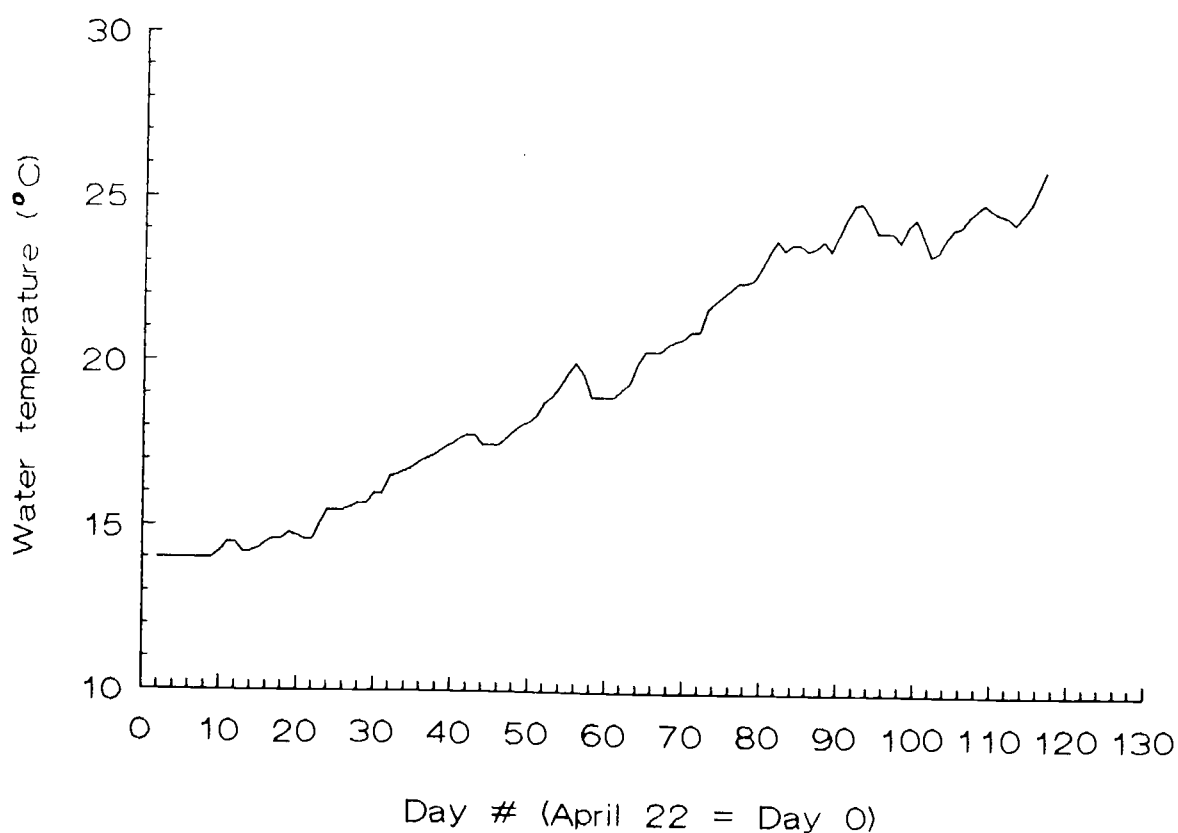


Figure II.15. Water temperature (in degrees celsius), from April 22 (day 0) to August 17 (day 117).

From the graph, we see that the water temperature remains 14°C during the last days of April. In May (day 9-39), the water temperature starts to rise, reaching 14.6°C on day 15. The latter part of May shows a further increase, up to 17.4°C. During the month of June (day 40-69), the temperature in the port of Stareso keeps rising; 19°C is recorded on day 64. July (day 70-100) shows a faster temperature rise, from 20.8°C to a peak of 25°C on day 93. After this day, the water temperature is seen to vary between 23.4 and 25°C. A quick rise, from 25 to 26°C, is observed from day 115 to day 117. After this day, temperature was no longer recorded as most males had deserted their artificial hole. Also, field research was at its end and research on morphological aspects of territorial males was conducted in the laboratory.

Comparing the data on the water temperature with data on the occupation of the artificial holes, we see that as the water temperature increases (and as day length increases), more *Blennius* males start to occupy a test-tube. During May, when water temperatures vary between 14 and 16°C, most *B.sphinx* males 'arrive' at the blocks. Only a few *B.incognitus* males start occupying a test-tube during this time. However, many more individuals of the latter species start occupying an artificial hole during the beginning of June, when water temperatures have reached 16°C.

II.F. NATURAL AND ARTIFICIAL HOLES

If we compare data on the occupation of the artificial holes with data obtained on the marked natural holes, we see that *B.incognitus* starts becoming territorial (starts occupying a hole) during May and June. Territorial males, occupying a natural hole, are seen to desert their nest earlier on in the season than males guarding an artificial hole; all 14 marked natural nests are unoccupied by August 8 (day 108), whilst all 28 artificial holes are deserted by August 28 (day 128).

The length of time that males were seen to occupy their hole ('guarding time'), was only estimated for males occupying a natural hole. In doing so, the assumption was made that males remain territorial in between successive observations. This has not been done for males occupying an artificial hole, as observations in front of the blocks were not regularly made towards the end of the season. Thus, a comparison of guarding time for males occupying a natural and males occupying an artificial hole can not be made.

Several 'ecological aspects', measured on and around the natural holes, may be compared with data on the artificial holes. The natural holes were found to vary in shape and size, as well as in their location to the surrounding area (see the summarised results of section I: natural holes).

The glass test-tubes were placed at similar depths, compared to the depths at which the natural holes were found, although the distance between the artificial holes was smaller than natural distances found between neighbours. The blocks were situated in a sunlit place, not highly exposed to waves, just like the marked nest sites. The test-tubes faced \pm NWW, with inclination angles between 0 and $+20^\circ$; most natural holes sloped upwards and faced between South and West. The entrance diameters of the different tubes compared well with the sizes of the 14 natural holes, although both types of test-tube had relatively large hole depths and hole volumes. Most natural holes had approximately round entrances.

B.incognitus was seen to use small and large sized test-tubes throughout the season; these must have been suitable in size and location, serving as a substitute for natural nest sites of this species.

* RESULTS *

III. MORPHOLOGICAL ASPECTS

SUMMARISED RESULTS

The morphological data obtained from 12 territorial *B.incognitus* males at the end of August, shows the following:

- Total length varies between 3.7 and 5.4 cm, the average is 4.3 cm (s.d.= 0.4, n = 12).
- Total body weight ranges from 0.49 to 1.27 g, the average is 0.85 g (s.d.= 0.239, n = 12).
- Total length and body weight are positively correlated (regression coefficient of +0.89).
- Territorial males have gonads weighing at least 0.003 g, but not more than 0.008 g. The average gonad weight is 0.0047 g (s.d.= 0.00142, n = 11).
- Body weight is not correlated to gonad weight (regression coefficient of +0.01).
- Relative gonad weight varies between 0.25 and 1.11 %. The average is 0.62 % (s.d.= 0.229, n = 11).
- Smaller, lighter territorial males tend to have relatively heavier gonads than larger, heavier males (regression coefficient of -0.44).

III. MORPHOLOGICAL ASPECTS OF TERRITORIAL MALES

Morphological data of 12 territorial *B.incognitus* males was obtained at the end of August. Appendix III.a shows the results on total length, body weight and gonad weight as well as the relative gonad weight (gonad weight expressed as a percentage over the total body weight), per individual.

Eight of the 12 males which were caught, had guarded artificial or marked natural holes. Four territorial males were caught from 'unobserved' nest sites which were situated in the quay wall. One of the 12 territorial males, the largest, was infested with round worms and its gonads could not be found in the body. Thus, only the total length and body weight of this large individual are included in the morphological data set.

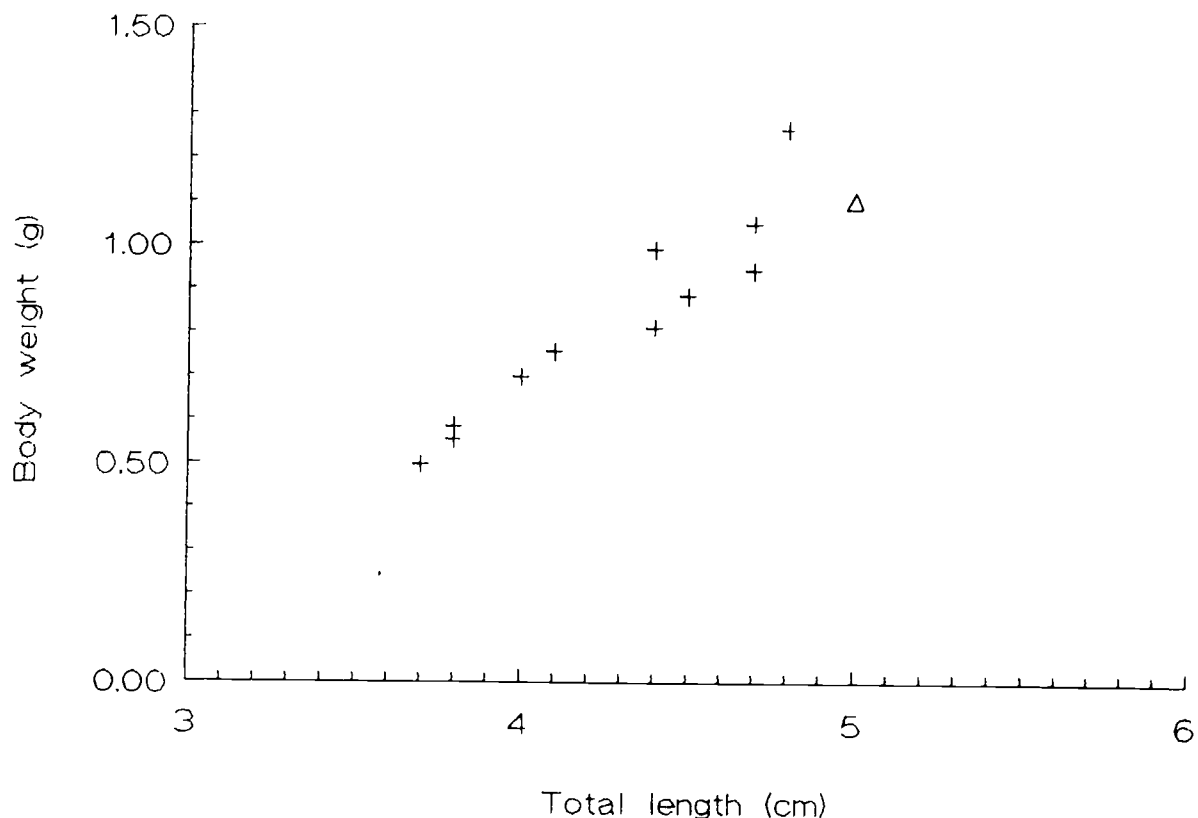


Figure III.1. Body weight (cm) against total length (cm), of 12 territorial *B. incognitus* males.

In Figure III.1, body weight (cm) is set against total length (cm) for 12 territorial *B. incognitus* males which were caught at the end of August.

The total length varies between 3.7 and 5.0 cm, the mean being 4.3 cm (s.d.= 0.4, n = 12). Body weight ranges between 0.49 and 1.27 g, the average being 0.85 g (s.d.= 0.239, n = 12). There is a clear correlation between these two parameters; the regression coefficient is +0.89.

Gonad weight (mg) of 11 territorial males is displayed in a frequency distribution graph: **Figure III.2.**

Gonad weight ranges from 0.003 to 0.008 g. Five ($\pm 45\%$) of 11 males have gonad weights between 0.003 and 0.004 g. The average weight is 0.0047 g (s.d.= 0.00142, n = 11).

Gonad weight (g) is set against body weight (g), for 11 territorial males, in **Figure III.3.**

Territorial males have a minimum of ± 0.003 g of gonads in their body. We see a negligible correlation (regression coefficient of +0.01) between gonad weight and body weight.

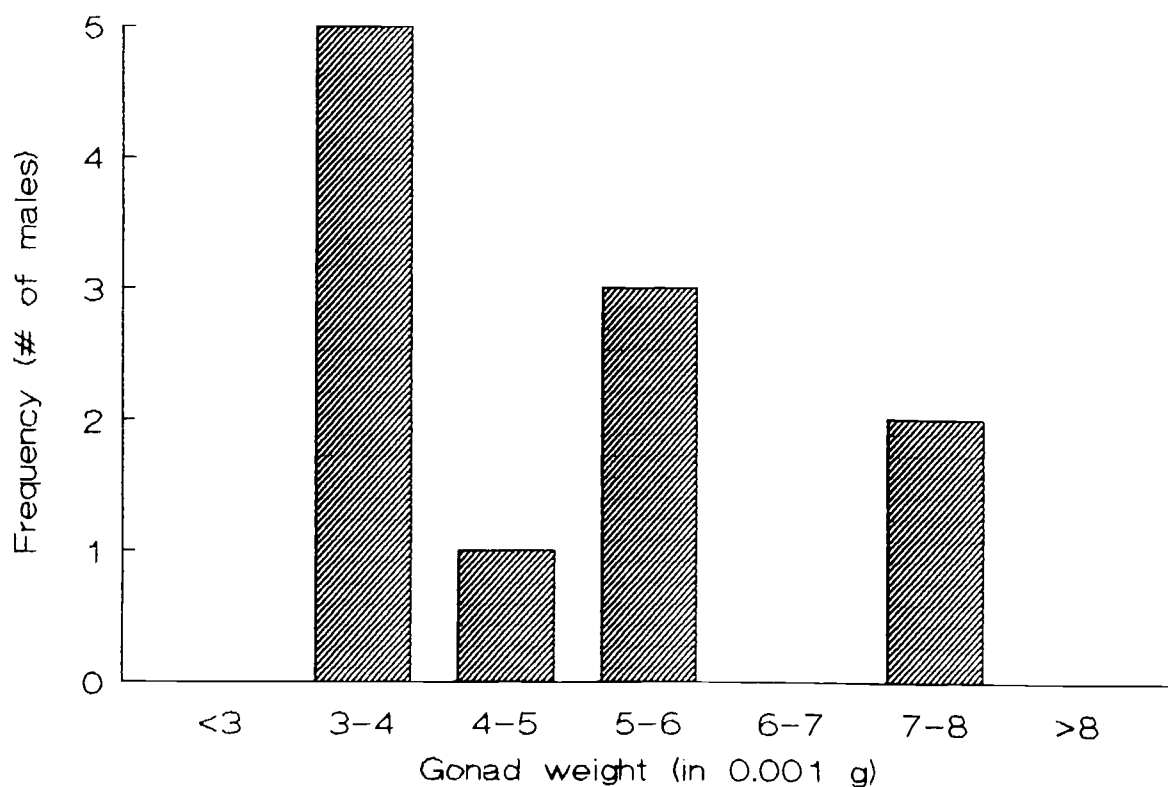


Figure III.2. Frequency distribution of gonad weight (mg), of 11 territorial males.

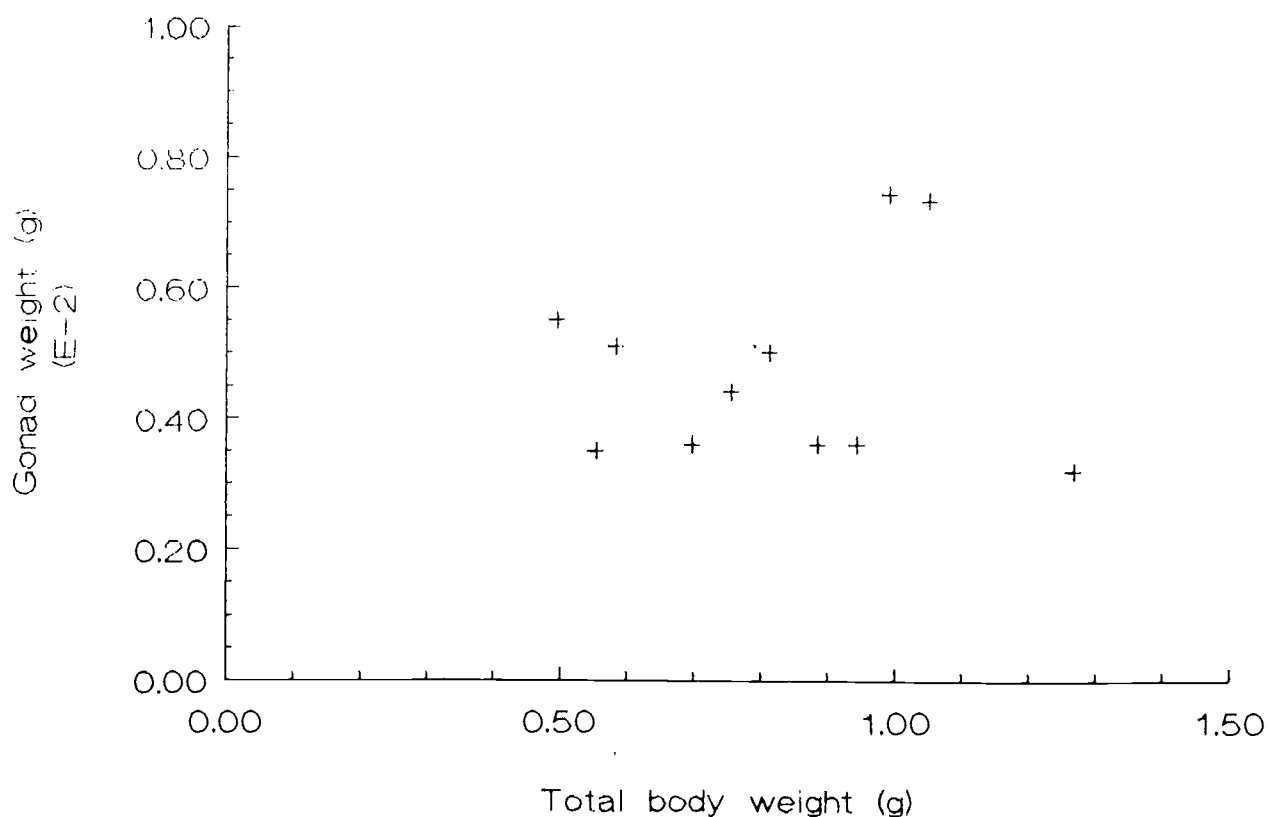


Figure III.3. Gonad weight (g) against body weight (g), of 11 territorial *B.incognitus* males.

Figure III.4 is a frequency distribution graph of relative gonad weight, (calculated as a percentage over the total body weight), for 11 territorial *B.incognitus* males at the end of August.

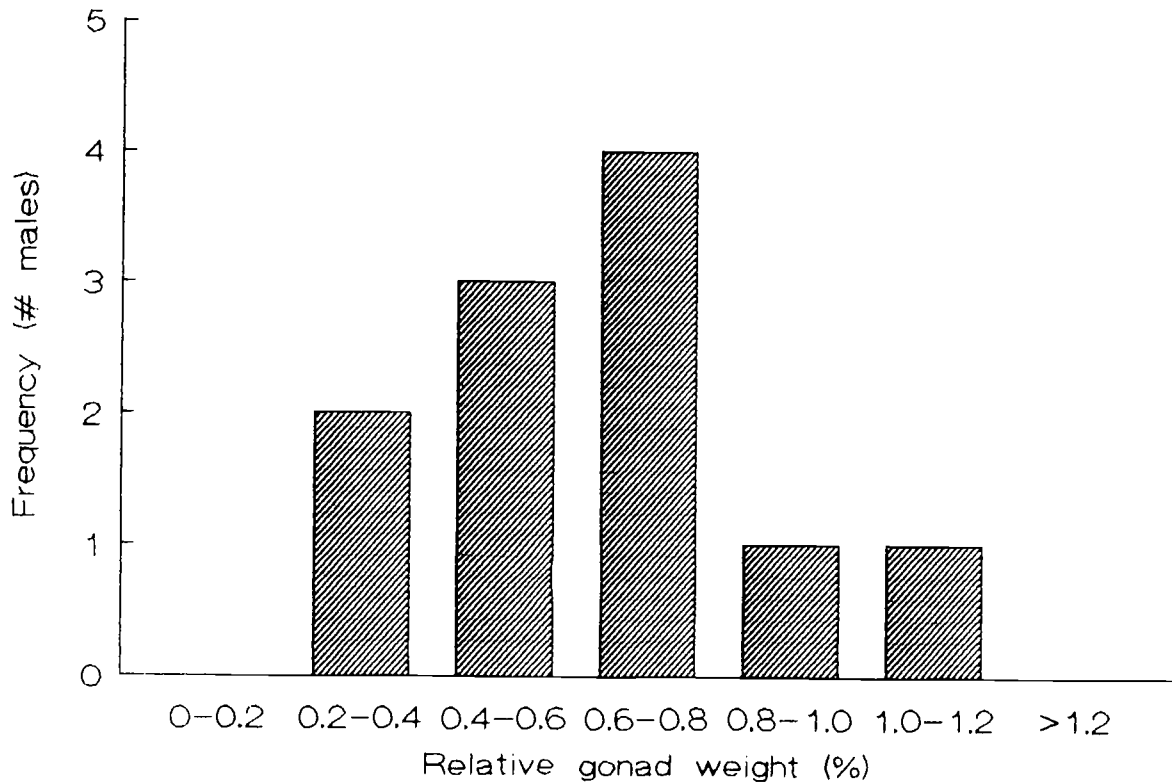


Figure III.4. Frequency distribution of relative gonad weight (in percentages), for 11 territorial males.

From the graph we see that relative gonad weight varies between 0.25 and 1.11 %. The modal class is 0.6-0.8 %. The average relative gonad weight, for 11 territorial *B.incognitus* males, is 0.62 % (s.d.= 0.229).

In Figure III.5, relative gonad weight (in percentages) is set against body weight (g). For 11 territorial males, a reasonable correlation (regression coefficient of -0.44) is found between relative gonad weight and body weight. Thus, at the end of August, smaller, lighter males tend to have relatively heavy gonads compared with larger, heavier territorial males.

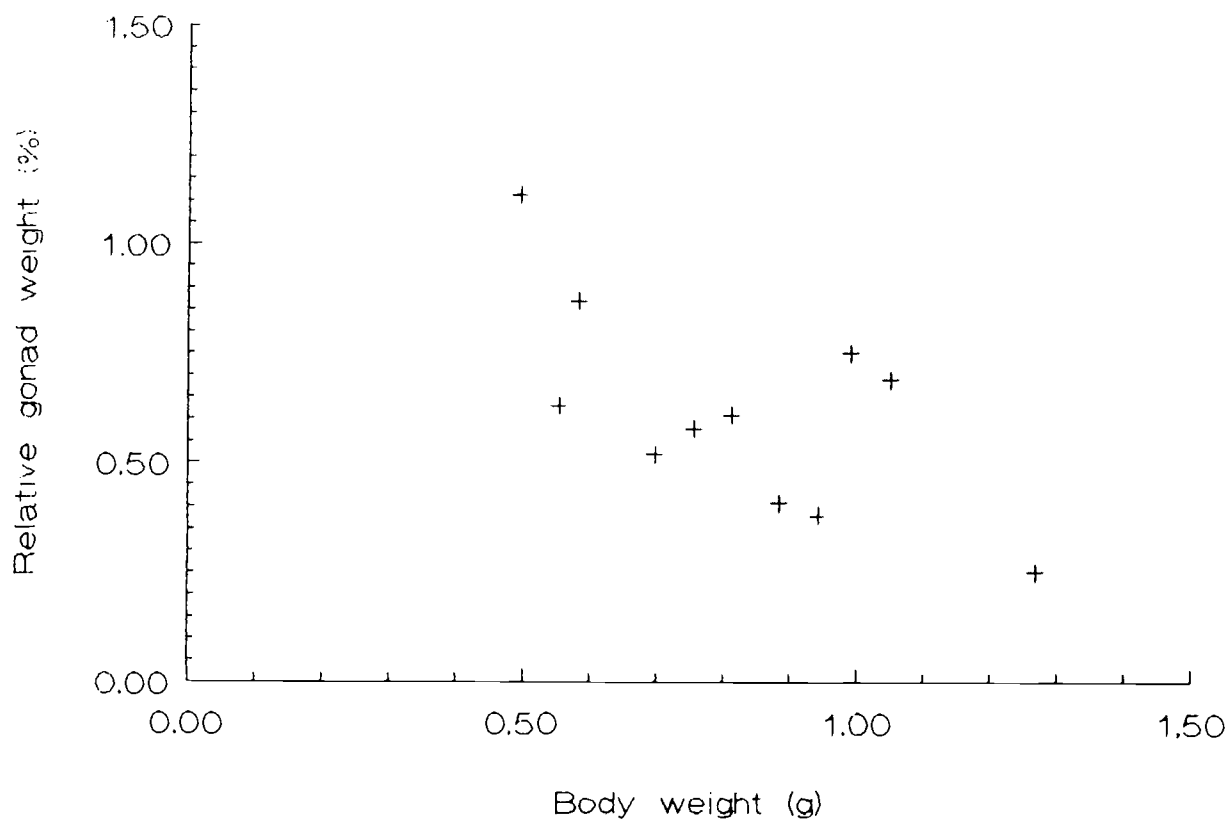


Figure III.5. Relative gonad weight (in percentages) against body weight (g), of 11 territorial *B. incognitus* males at the end of the season.

* RESULTS *

IV. BEHAVIOURAL ASPECTS

SUMMARISED RESULTS

Qualitative observations suggest that territorial males swim, feed and scratch themselves most often during the afternoon. Courtship behaviour and spawning is seen before midday, as well as most agonistic behaviours, directed towards conspecific males. Resting is observed throughout the day, as well as ventilating the nest and the occasional interaction with another individual (of the same, or a different species).

Quantitative observations confirm the above qualitative results. Per daytime category (see materials and methods), the duration of each state is summarised and expressed as a percentage over the total protocol time of the corresponding category; the frequency of each event is expressed per hour of total protocol time, for each category:

State 1 (swimming); ranges from 0.06 to 2.19 %. This state is rare during late morning and is mostly seen after 12:00 hours.

State 2 (resting outside or partly inside the nest); varies between 41.19 and 71.82 %. It occurs in similar amounts of time during the 5 daytime categories.

State 3 (ventilating the hole); ranges from 8.23 to 17.6 %. Comparable amounts of time are spent on fanning the eggs throughout the day, the maximum time being spent during the evening.

State 4 (agonistic display); varies between 0.04 and 0.66 %. It is least seen during the afternoon and mostly recorded during the evening.

State 5 (courtship display); ranges from 0.02 to 0.65 %. It is rarely seen after 15:00 hours. Most courtship display is seen between 9:00 and 12:00 hours.

State 6 (totally inside hole); ranges from 16.44 to 39.5 %. It is mostly seen during the evening, but accounts for similar amounts of time during the rest of the day.

State 7 (interspecific interaction); varies between 0.07 and 0.17 %. It is least seen during the early morning, but throughout the rest of the day, similar amounts of time are spent on this state.

States 8, 9 and 10 (intraspecific interactions); each takes very little time during the day. The total time spent on these states, ranges from 0 to 1.19 %. Intraspecific interaction is not seen after 15:00 hours.

- Event 1 (leaves hole); occurs between 2 and 6.5 times, per hour. It is most frequent during the afternoon.
- Event 2 (changes guarding position); has frequencies ranging from 50.6 to 80.9, per hour. It occurs in similar numbers throughout the day.
- Event 3 (trembles body); varies between 0 and 5.9 times per hour. Males are not seen to tremble after 12:00, most is observed between 9:00 and 12:00.
- Event 4 (nips the vegetation); is encountered 9.5 to 26.8 times per hour. Males mostly feed during the afternoon and evening.
- Event 5 (scratches body); is seen to occur between 0 and 3.7 times per hour. Most scratching is seen during the late afternoon, none is recorded in the evening.
- Event 6 (female enters hole); occurs between 0 and 0.8 times per hour. Most females enter a hole during the early morning hours. Event 6 is not seen to occur after 12:00 hours.
- Event 7 (female leaves hole); occurs between 0 and 1.4 times per hour. Highest frequencies are found during early morning. Event 7 does not occur after 15:00 hours.

IV.A. QUALITATIVE RESULTS

Qualitative results on behaviour were gained by hanging still in front of holes being guarded by *B.incognitus* at different times during the day, taking notes on a PVC slate. The different non-social and social activities displayed by territorial *B.incognitus* males could be recognised and described.

IV.A.1. NON-SOCIAL BEHAVIOUR

SWIMMING

Like all other blennies, *B.incognitus* lacks a swim bladder. It stays close to the substrate and does not swim far unless it chases away intruders or is being chased away itself. Typical swimming movements consist of short hops from one place to another, interspersed with rests on the substrate.

FEEDING

B.incognitus, having cutting teeth in a row, is able to bite pieces out of larger clumps of food. When feeding, males take quick bites ('nips') out of the algal covering of the substrate. Usually males leave their nest to feed, but occasionally the territory owner remains (partially) inside its hole, nipping the algae around the hole entrance.

SCRATCHING

B.incognitus individuals are seen to scratch (scrub) themselves, outside the nest site. They do this by turning onto one side, starting to swim with a tail beat that rubs their body against the substrate. Scratching lasts only a very short time.

RESTING

When no other outward activity is apparent, *B.incognitus* is assumed to be 'at rest'. Outside of its hole, the male rests by propping up the anterior part of its body on its pectoral and ventral fins. Swimming is frequently interspersed with such rests. Inside their hole, guarding males are often seen to rest, pausing from ventilating the nest with their lateral fins. *B.incognitus* is active during the light period. Before dawn and after dusk, individuals are seen to retrieve into shelters to rest during the dark period.

Qualitative observations on NON-SOCIAL behaviours show that territorial males swim, feed and scratch themselves most often during the afternoon. Resting is observed throughout the day.

IV.A.2. SOCIAL BEHAVIOUR

IV.A.2a. Agonistic behaviour

B.incognitus males are territorial during the reproductive period, lasting from May until August. During this time, males occupy and guard a hole. Agonistic behaviour is usually directed towards other *B.incognitus* males, but occasionally towards females of the same species or individuals of other species.

When an 'intruder' approaches the nest of a territorial male, the latter usually responds with 'agonistic display'. The territory holder moves forwards, leaving only its tail inside the nest and raises its head and the anterior part of its body towards the intruder. The unpaired fins are spread, especially the dorsal fin. This 'back-arch' (or 'reared-up') position, combined with the spread fins, was recorded as agonistic display.

B.incognitus males are not very aggressive and usually such agonistic display is enough for intruders to leave the immediate area surrounding the nest. When this is not the case and an intruder stays or moves closer to the nest, the territory holder is often seen to surge forward out of its nest, attacking and biting the intruder. A fight may result, the 2 individuals biting each other vigorously until one individual gives up and is chased away.

If a territory owner feels intimidated by the presence of an intruder, it does not attack but is seen to retreat deeply into its nest. The intruder may attempt to bite the hiding owner but this usually fails and after some time, the intruder is usually seen to leave.

INTRASPECIFIC INTERACTION : MALE - MALE

Agonistic behaviour as described above, was most frequently observed between two *B.incognitus* males. In all of the observations, the territory holder was never seen to 'lose the match' from a conspecific male intruder.

INTRASPECIFIC INTERACTION : MALE - FEMALE

When females approached a nest, *B.incognitus* males did not always respond with courtship display. On several occasions, females were ignored by resident males, even when these were not (already) occupied with another female. Sometimes, females were nipped at and chased away. This behaviour was often seen just after a female had spawned inside the nest. Agonistic display was rarely directed towards conspecific females species.

INTRASPECIFIC INTERACTION : FEMALE - FEMALE

B.incognitus females usually ignore each other. A female can be seen to wait outside a nest, whilst another female is busy spawning inside the nest.

On two occasions, two females were seen in agonistic interaction with each other, close to a nest. The females approached each other with raised heads and nipped and chased each other. On both occasions, the resident of the hole displayed courtship behaviour, such that one of the females was attracted into the nest. Shortly after the first female and the male had entered the nest, the second female shot into the nest as well, disrupting the pair, causing all three individuals to leave the hole. The two females chased each other away, whilst the male was left alone to guard its nest. During these observations, the sex of each individual was 'determined' by outward appearance only.

INTERSPECIFIC INTERACTION

B.incognitus males are not very aggressive towards other species. However, when a *Tripterygion* approaches a nest, agonistic display is shown by the territory holder. This behaviour is also shown when other (usually male) *Blennius* species (*B.sphinx*, *B.zvonimiri* and *B.canevae*) approach the nest.

On one occasion, a large *B.sphinx* male was found dead, with its head stuck in a small sized test-tube, which had been occupied by *B.incognitus*. The owner had probably fled before the intruding *B.sphinx* male got stuck in the hole, head first. Also, on one occasion, a test-tube which was occupied by *B.sphinx* was taken by *B.incognitus*. This lasted for a couple of days, after which *B.sphinx* was again seen to be the test-tube resident.

B.incognitus males are not seen to react aggressively towards larger species of fish, such as wrasses. When such fish pass close by a nest, the owner often retreats into its shelter for a short period of time.

IV.A.2b. Sexual behaviour

During the reproductive season (May to August) *B.incognitus* males are territorial, occupying and guarding a haptic hole which serves as the spawning site for several females.

Approaching females may be completely ignored by territorial males, but usually males respond with courtship display; the male raises the anterior part of its body, keeping only its tail inside the nest, and spreads its dorsal fin (back-arch position). The spread dorsal fin in the reared-up position are thus used in an agonistic and in a sexual context. However, during courtship display, the male repeatedly trembles (quivers) its body in this position. The female reacts to this display by coming closer to the nest, until she is next to the hole entrance. The courting male is sometimes seen to move one of its lateral fins up and down, close to the female. Courtship display usually takes less than a minute; the male then pops out of its hole, occasionally jumping over or against the waiting female. Whilst the male waits, the female enters the nest, usually head first.

Usually, a female seems to 'inspect' the hole, after which her head appears in the entrance. The female may leave the nest without depositing her eggs or retreat into it again. In case of the latter, the male also enters the nest, tail first, to position itself beside or in front of the female. If the hole is big enough for the two individuals, the spawning pair remain in the nest until the female finishes her egg deposition. The male is seen to tremble once in a while, probably fertilizing the eggs during this time. Sometimes, a nest is too small for two blennies and, in such a case, the male will wait outside the hole (usually after several failed attempts to enter it).

Table 2 shows the times (during the day) in which females were seen to enter and/or leave a nest, as well as the amount of time (in hours, minutes and seconds) that they had (at least) spent inside a nest.

Out of 12 observations, the latest time at which a female is seen to enter a nest, is 10:01 (09-6; nest 2). The latest time at which a female is observed to leave a nest, is 12:14 (15-7; nest 11). Thus, we see that spawning is concentrated during the morning hours; after 12:15, no more females are seen to enter or leave a nest. The amount of time that a female spends inside a nest, varies between a few seconds (female apparently rejects the nest and does not spawn) to several minutes. Forty minutes is the maximum amount of time a female is seen to spend inside a nest.

Table 2. Time during the day when females enter and/or leave the nest of a territorial male, as well as the (least) amount of time they spend inside a hole.

		- TIME -				Duration	
Date	Hole	Protocol starts	Female enters	Female leaves	Protocol ends	of female in nest	
=====							
(PVC slate):							
22-5	11.3	8:00:			8:30:	0:30:	+
09-6	n2		10:01:	10:41:		0:40:	
20-6	n2		9:15:	9:44:		0:29:	

(Event-recorder):							
05-7	n2	8:15:		8:15:19		0:00:19	+
	n2		8:19:27	8:26:52		0:07:25	
	n2		8:32:10		8:45:18	0:13:17	+
05-7	n11	8:50:		8:59:15		0:09:15	+
	n11		9:10:52	9:42:28		0:31:36	
08-7	n11	9:55:		9:55:53		0:00:53	+
	n11		9:56:45	9:56:48		0:00:03	
15-7	n11	12:04:		12:14:28		0:10:28	+
15-7	n11		6:40:31		6:50:20	0:09:49	+
=====							

Times are expressed in hrs:mins:secs

Durations marked with '+' : female was not seen to enter & leave nest

Territorial males that had just started to occupy an artificial hole, often 'received' eggs after only a couple of days. Some males received very little eggs, even when they occupied their nest over a long period of time. Females deposited their eggs at the far end of the test-tube first, and these were usually stuck onto the glass in little groups. Subsequent clutches, from the same or other females, were usually placed next to earlier ones, thus filling the tube from the back to the front.

After spawning, a leaving female was often nipped at by the resident male and occasionally she was chased away from the nest. The territory holder was left to take care of the eggs until they hatched.

IV.A.2c. Parental care

Parental care is equal to paternal care in *B.incognitus*, as this is solely done by territorial males. A territory holder defends its hole, containing eggs, against intruders. The male may also clean the eggs (from algae or bacteria). It regularly fans the clutches by waving its lateral fins up and down, thus ventilating the nest. Fanning (ventilating) is usually done whilst about a quarter or half the length of the male's body protrudes from the hole.

B.incognitus individuals may eat a few of their 'own' eggs, as well as eggs of other *Blennius* species. Numbers of eggs present in the test-tubes occasionally decreased, as time (and females) passed by. On the occasion that a *B.incognitus* male took over a test-tube which was first guarded by *B.sphinx*, the yellow coloured 'sphinx eggs' had soon disappeared. These eggs were probably eaten by the territorial *B.incognitus* male or by conspecific females that came to spawn their own reddish-brown coloured eggs in the same tube. After a few days, the same tube was re-taken by *B.sphinx* and the '*B.incognitus* eggs' disappeared in a similar fashion.

Qualitative results on the different SOCIAL behaviours show the following:

Territorial *B.incognitus* males interact with other fish throughout the day. During the morning hours, males display courtship behaviour, attracting females to spawn inside their nest. During these hours, intruders are chased away from the nest more vigorously than later on in the day. Such agonistic behaviour (interaction) is usually seen between males of the same species. Territorial males are seen to ventilate their nest regularly, throughout the day. During the afternoon, males return to their nest in between feeding, to fan the eggs.

IV.B. QUANTITATIVE RESULTS

The event-recorder was used to gain quantitative data on the different behaviours shown by territorial *B.incognitus* males. Protocols were divided into 5 categories, according to their 'starting time', irrespective of the male which was observed or the time during the season at which observations were recorded. The five daytime categories (each spanning 3 hours) into which the protocols were divided, also listed in the materials and methods, are the following:

06:00 - 09:00 hours: early morning
09:00 - 12:00 hours: late morning
12:00 - 15:00 hours: early afternoon
15:00 - 18:00 hours: late afternoon
18:00 - 21:00 hours: evening

A list of all the protocols made between July 5 (day 74) and August 9 (day 109), divided into the 5 daytime categories, is given in **Appendix IV.a**. 'True protocol time' (in minutes and seconds) is also given; this is the duration of a protocol after the duration of the first and last 'incomplete' states has been subtracted (thus only the durations of fully completed states are included).

IV.B.1. STATES

For each state, the total duration was summarised per daytime category and this was expressed as a percentage over the total (true) protocol time of the specific category: this data is given in **Appendix IV.b**. The total protocol time for a category was obtained by summarising all true durations (without start and end states) of the protocols which were divided into that category.

The 'lost' (out of site) key (state 11) was rarely used, as territorial males usually swam and fed close to their nests so that they could be continually observed. Durations of this state have not been included in true protocol times.

Figure IV.1 shows the share of total protocol time (in percentages) per daytime category, in which state 1 ('swimming') is recorded. Swimming was recorded when a male moved from one place on the substrate to another. The rests in between the 'hopping' movements were not recorded as state 1, but as 'resting' (state 2).

From the pie chart, we see that swimming does not account for a large part of total protocol time, throughout the day; it takes up 0.06 to 2.19 % of the total protocol of a category. Territorial *B.incognitus* males mostly swim during late afternoon (between 15:00 and 18:00 hours). Swimming is rarely seen during late morning (between 9:00 and 12:00 hours).

Figure IV.2 shows the share of total protocol time (in percentages) for the five daytime categories, in which males are seen to 'rest' (state 2). Resting was recorded in between swimming movements as well as at times when a male seemed to be inactive inside its nest (its head and usually part of its body protruding out of the hole).

In the figure, we see that territorial males spend a large amount of time during the day doing 'nothing'. The duration of such inactivity varies between 41.19 % of the total protocol time during evening to 71.82 % of the total protocol time during early afternoon.

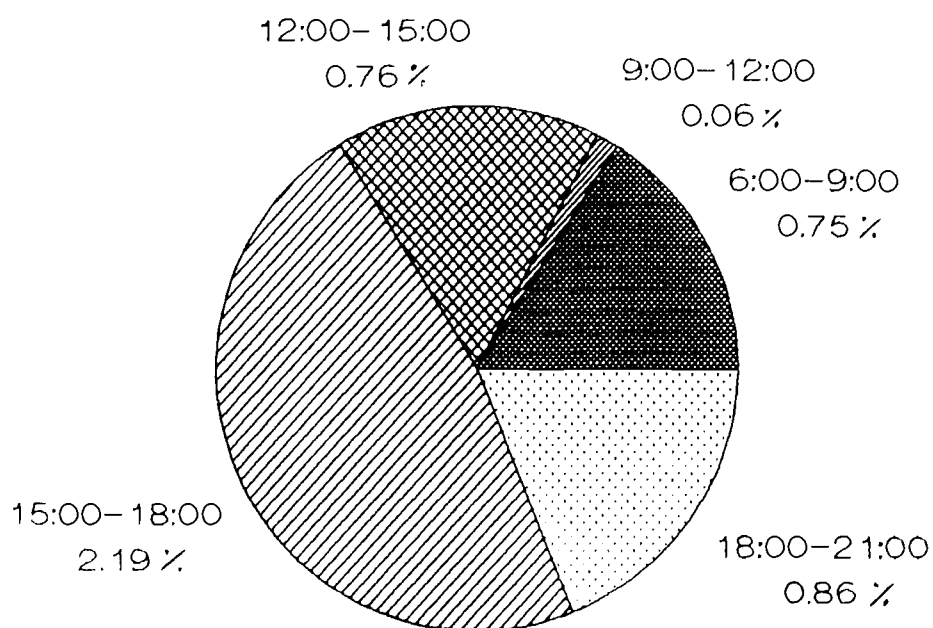


Figure IV.1. Time spent on state 1 (swimming), expressed as a percentage over total protocol time, per category.

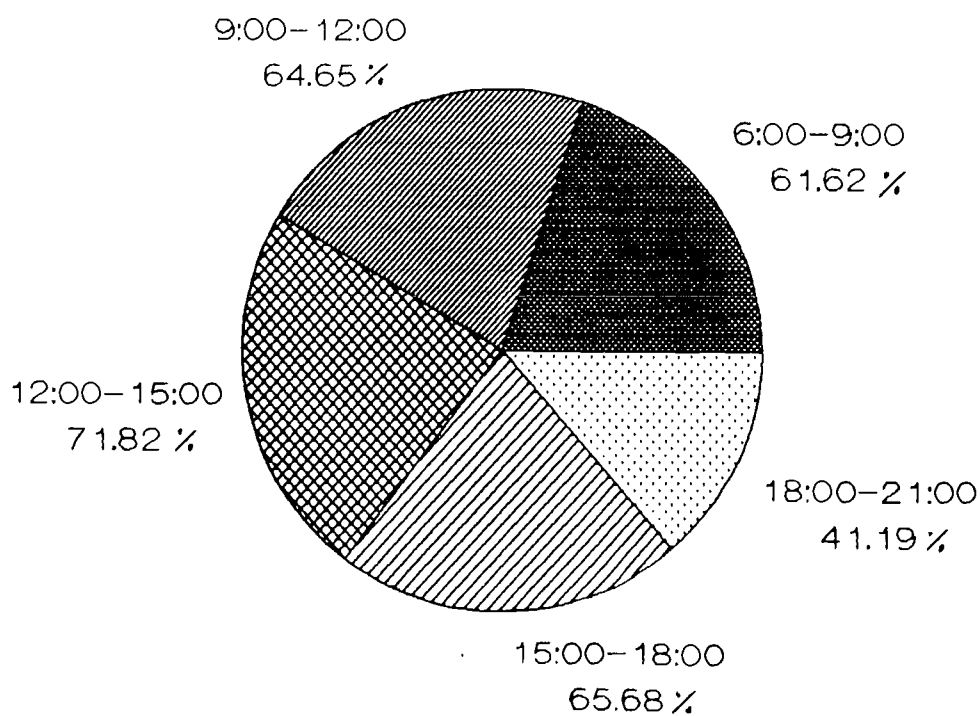


Figure IV.2. Time spent on state 2 (resting), expressed as a percentage over total protocol time, per category.

Figure IV.3 shows the amount of total protocol time (in percentages) in which territorial males are seen to 'ventilate' their nest (state 3) during the day. 'Fanning' was registered when a territorial male visibly waved one or both of its lateral fins up and down, to ventilate the hole. Usually, fanning is done with about half the length of the male's body protruding out of the hole.

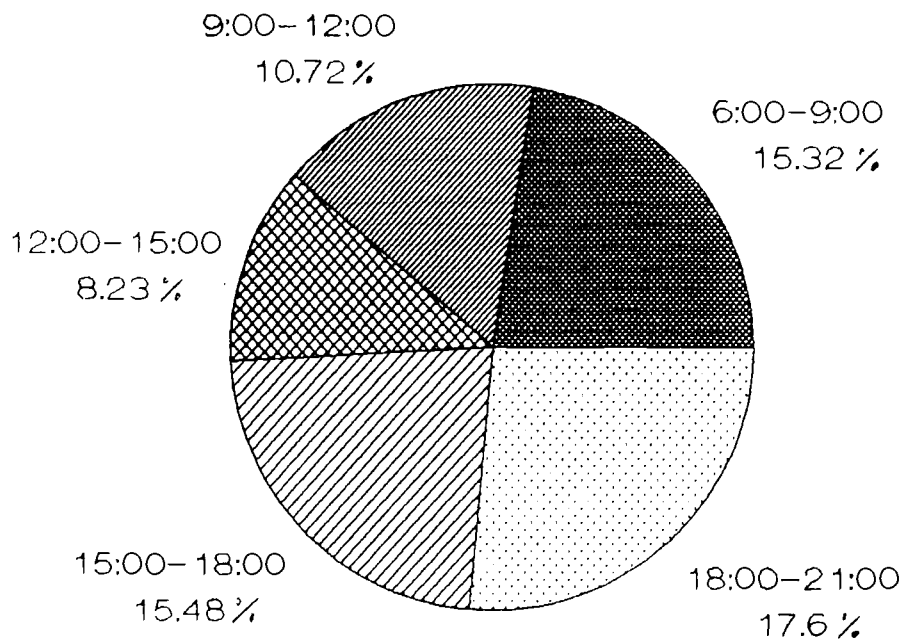


Figure IV.3. Time spent on state 3 (ventilating), expressed as a percentage over total protocol time, per category.

From the figure, we see that territorial males spend a reasonable amount of time ventilating their haptic hole. Fanning percentages do not differ greatly between the 5 daytime categories. Territorial *B.incognitus* males spend between 17.6 % (evening) and 8.23 % (early afternoon) of total protocol time ventilating their hole.

Figure IV.4 shows the share of total protocol time (in percentages) per daytime category, in which males are observed in state 4 ('agonistic display'). Agonistic display has been described in section IV.A.2a. This state was not recorded when the territory holder was physically interacting (i.e. biting, fighting) with an intruder, but only when the male displayed the back-arch position with spread fins.

The low percentages in the pie chart show that males spend very little time on agonistic display throughout the day. This ranges from 0.04 % of total protocol time during early (and late) afternoon, to 0.66 % of total protocol time during evening. Although most agonistic display is observed between 18:00 and 21:00 hours, males apparently 'ignore' intruders during the afternoon.

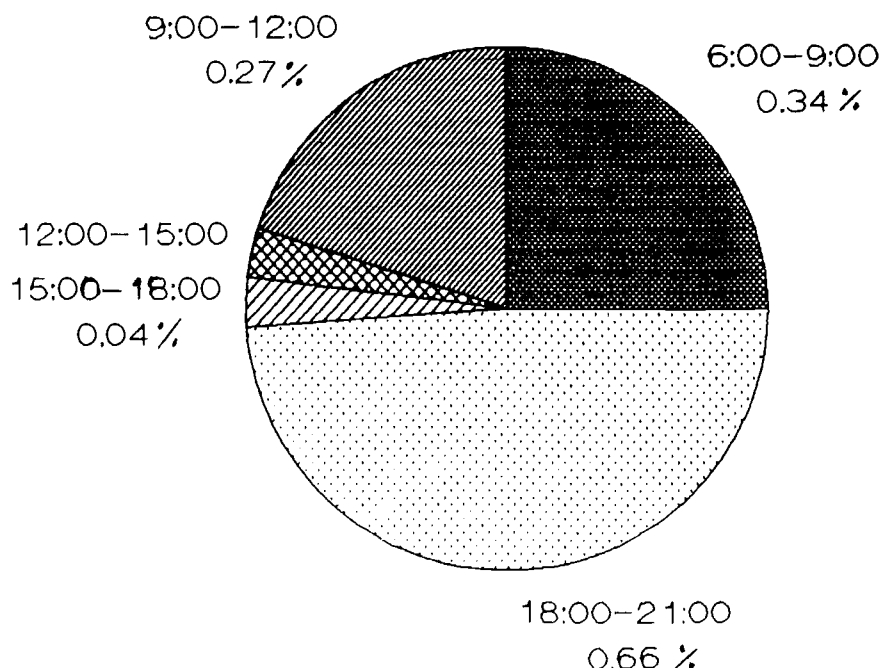


Figure IV.4. Time spent on state 4 (agonistic display), expressed as a percentage over total protocol time, per category.

Figure IV.5 shows the amount of total protocol time (in percentages) per category, in which males are seen to display courtship behaviour (state 5). 'Courtship display' was recorded in a similar fashion to agonistic display; it includes the back-arch or reared-up position combined with spread fins, interspersed with quivering. Courtship display was directed towards conspecific females, which usually responded by drawing nearer to the nest. From the pie chart, we see that courtship display takes up only a very small part of the total protocol time of each daytime category. The low percentages range from 0 % during evening, to 0.05 % during late morning. *B.incognitus* is mostly seen to court females during the morning, especially between 9:00 and 12:00 hours. Males are rarely seen to display courtship behaviour after 15:00 hours.

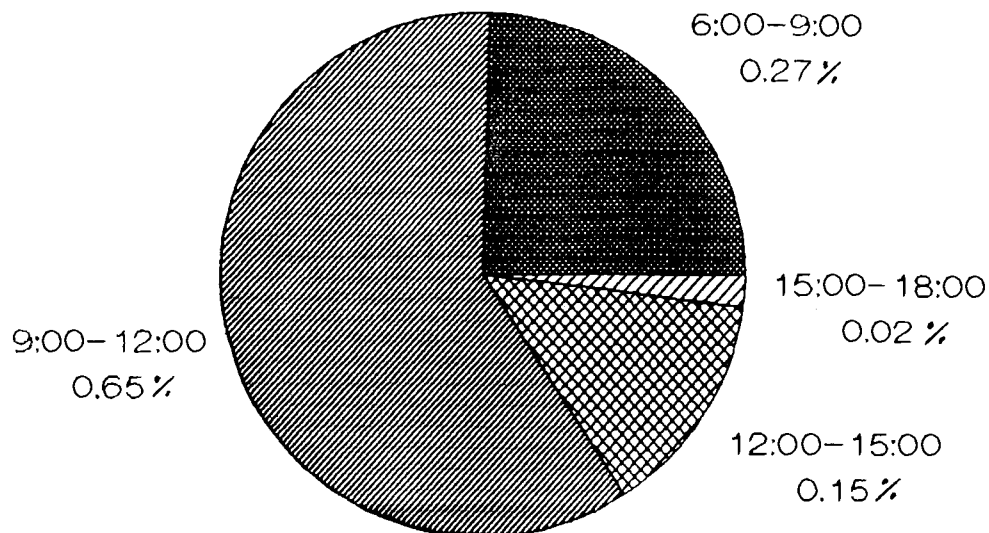


Figure IV.5. Time spent on state 5 (courtship display), expressed as a percentage over total protocol time, per category.

Figure IV.6 shows the share of total protocol time (in percentages) per daytime category, in which males were 'totally inside' their nest (state 6). Males could be seen to move about inside their hole, but the type of activity they displayed could not be recognised. State 6 was thus recorded when males were practically out of site, having retreated into their hole. Occasionally, territorial males are seen to dart out of their nest, following this with an immediate U-turn, to enter it again, head first. This quick behaviour has been included in state 6.

The amount of total protocol time per daytime category, in which males are totally inside their nest, varies between 16.44 % during late afternoon and 39.50 % during evening. Thus, territorial *B.incognitus* males spend a fairly large part of their time inside the nest, throughout the day.

Figure IV.7 shows the amount of total protocol time (in percentages) per daytime category, in which males interact with an individual of another species (state 7). This 'interspecific interaction' does not include agonistic display (state 4). State 7 was recorded as soon as an intruder responded to the agonistic display of the territory owner, by becoming engaged in a fight with the territory owner or by being chased away by the territorial male. From the pie chart, we see that interspecific interaction takes up very little of the total protocol time of each daytime category. Percentages of time spent in this state are similar per category, varying between 0.07 % during early morning to 0.19 % during evening.

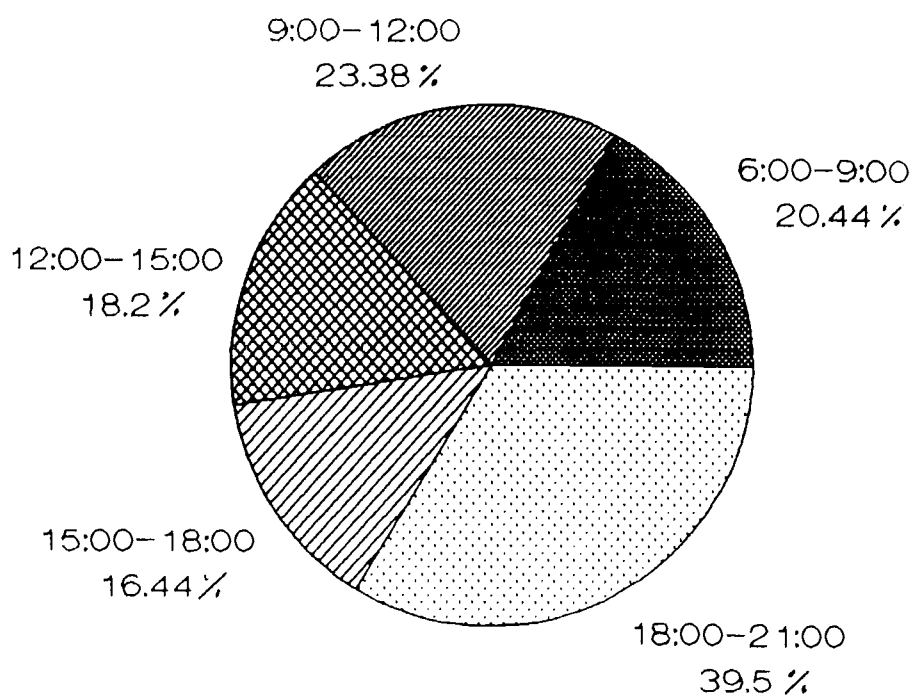


Figure IV.6. Time spent on state 6 (totally inside hole), expressed as a percentage over total protocol time, per category.

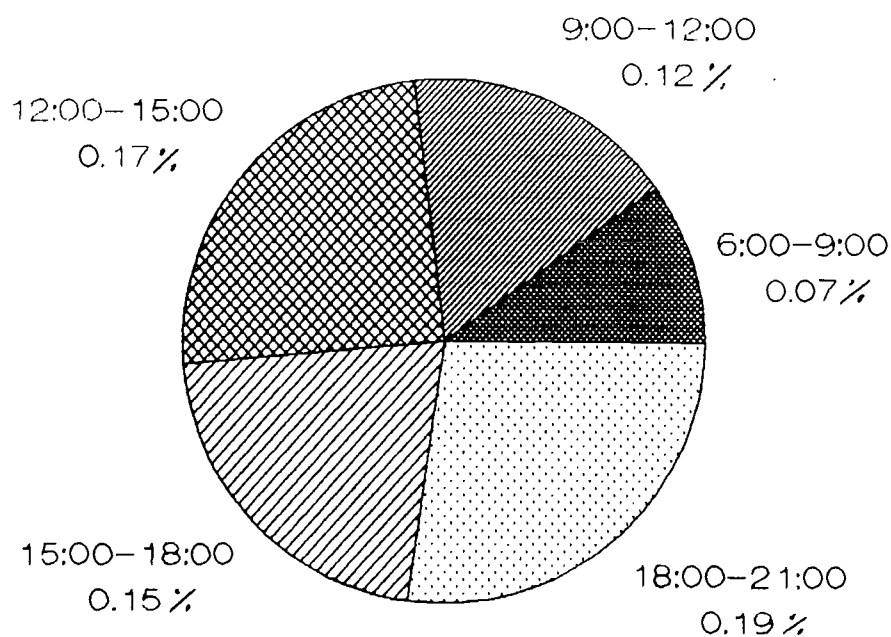


Figure IV.7. Time spent on state 7 (interspecific interaction), expressed as a percentage over total protocol time, per category.

The total duration (in percentages) per category spent on 'intraspecific interaction' is displayed in Figure IV.8. This graph includes 3 types of intraspecific interaction; state 8 for male-male interaction, state 9 for male-female interaction and state 10 for intraspecific interaction when the sex of the second individual was uncertain. 'Intraspecific interaction' does not include agonistic or courtship display. It includes all other types of more physical interaction (i.e. biting, fighting, chasing) observed between two *B.incognitus* individuals.

From the graph, we see that each type of intraspecific interaction takes up only a very small part of the total protocol time of a category. The total amount of time spent on intraspecific interaction (states 7, 8 and 9) is low, varying between 0 % of total protocol time during late afternoon and 1.19 % during early morning. Territorial males interact with females during the early morning hours. We cannot say how many females were not recognised so that these interactions were included in state 10. Male-male interaction is most frequent during early afternoon, but is also found during late morning. Here, also, we do not know which part of state 10 is male-male interaction.

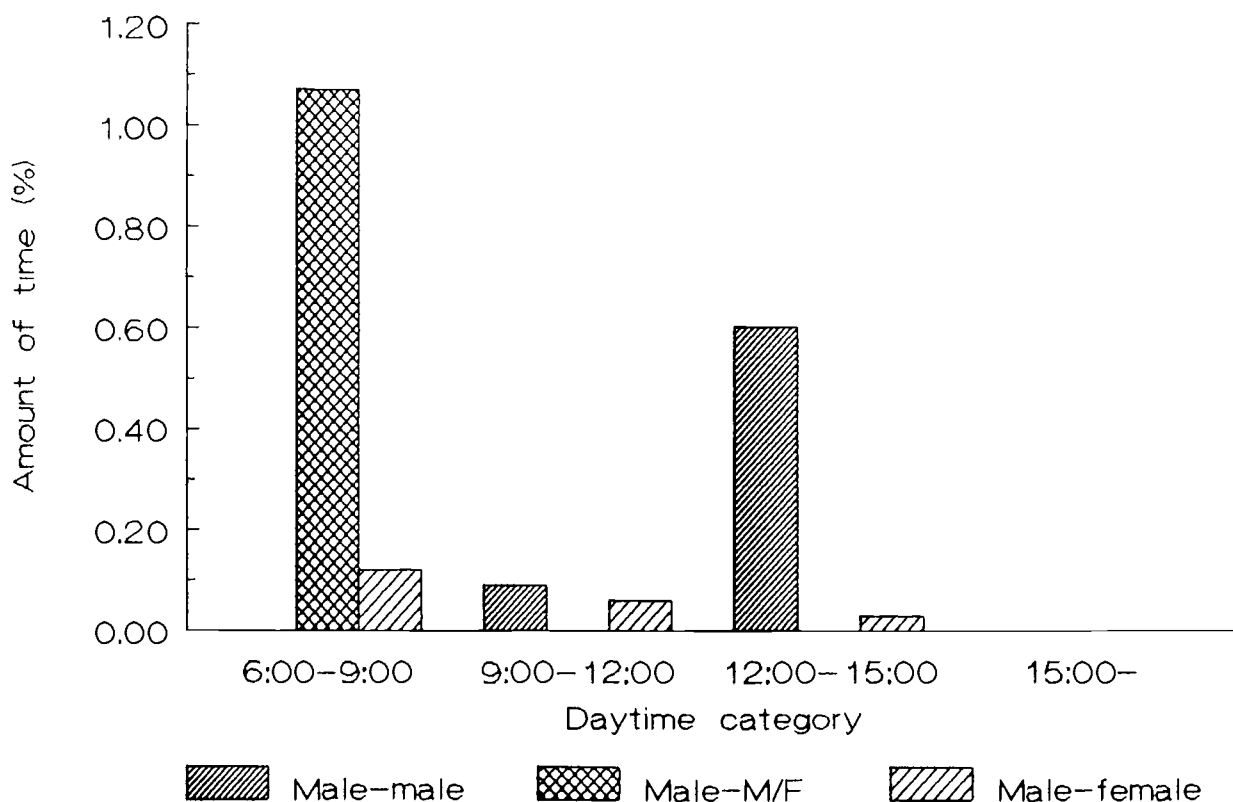


Figure IV.8. Time spent on state 8, 9 and 10 (intraspecific interaction), expressed as a percentage over total protocol time, per category.

IV.B.2. EVENTS

The data on the different events (obtained over the whole research period) was assimilated per daytime category in a similar fashion to the data on the states. For each category, the number of times an event had occurred was summarised and expressed per hour of total (true) protocol time for that specific category. In doing this, events which were recorded during the first or last incomplete state of a protocol were not included. In the figures that follow, the summarised frequencies of the different events are expressed per hour of total protocol time of each category. This data is also shown in **Appendix IV.c**.

Figure IV.9 shows the number of times (per hour of total protocol time) that territorial males are seen to leave their nest (event 1), for each of the five categories. 'Leaving the nest' was recorded as soon as the total length of the male's body had left the hole (and the male did not immediately return into it with a fast U-turn, as this was included in state 6).

We see that the number of exits per hour of total protocol time, varies between 1.7 during evening and 6.5 during early morning. The males mostly leave their nest between 6:00 and 9:00, as well as during the afternoon hours.

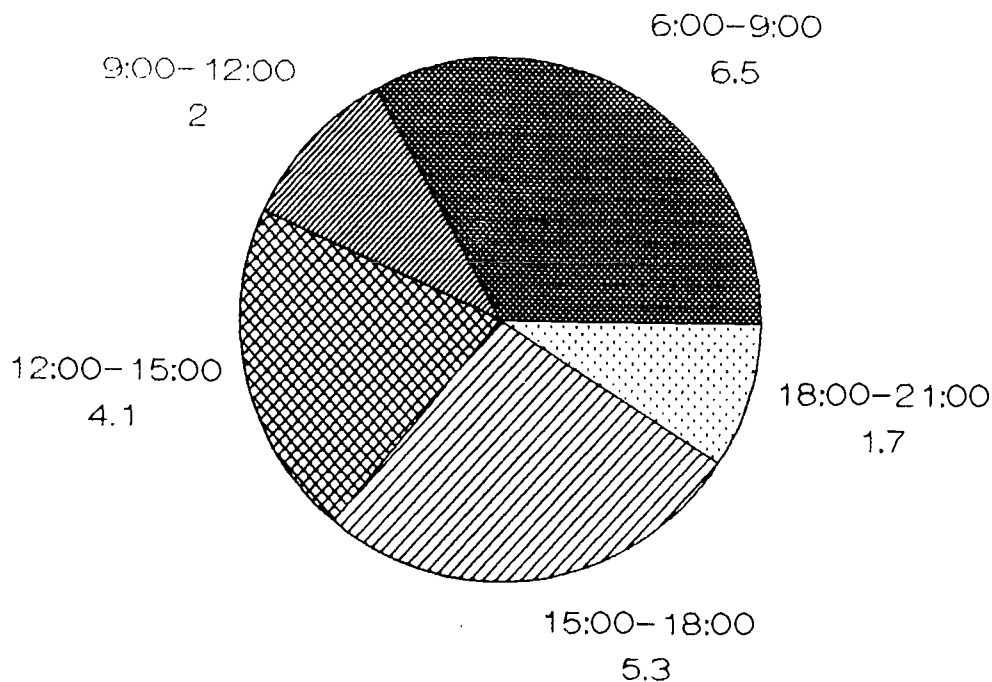


Figure IV.9. Frequency of event 1 (leaves hole), expressed per hour of total protocol time, per category.

Figure IV.10 shows the number of times (per hour of total protocol time), that males are seen to change their 'guarding position' (event 2), for each of the daytime categories. Changes between the following three different 'guarding positions' were registered as state 2;

- 1/4 out = head only, protrudes from hole
- 1/2 out = head and first part of body, including lateral fins, protrude from hole
- 3/4 out = head and large part of body, including dorsal fin, protrude from hole

From the pie chart, we see that males change their guarding position very often during each of the five categories. The number of changes varies between 50.6 per hour during evening, to 80.9 per hour during late morning.

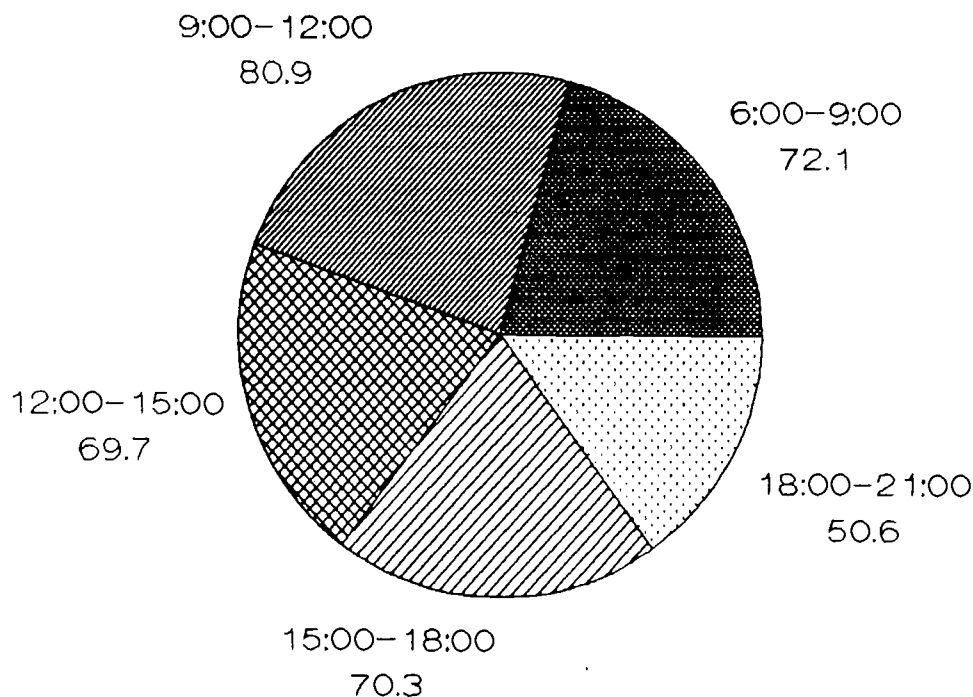


Figure IV.10. Frequency of event 2 (changes position), expressed per hour of total protocol time, per category.

Figure IV.11 shows the number of times (per hour of total protocol time) that males 'quiver' their body (event 3), for each category. Trembling is displayed in the reared-up position, with spread fins, whilst courting a female as well as during the time a female is spawning in the nest. We see that trembling occurs in low frequencies during each of the daytime categories. Frequencies range from 0 to 5.6, per hour of total protocol time for a category; it is not seen after midday. This event is most frequently displayed between 6:00 and 9:00 hours.

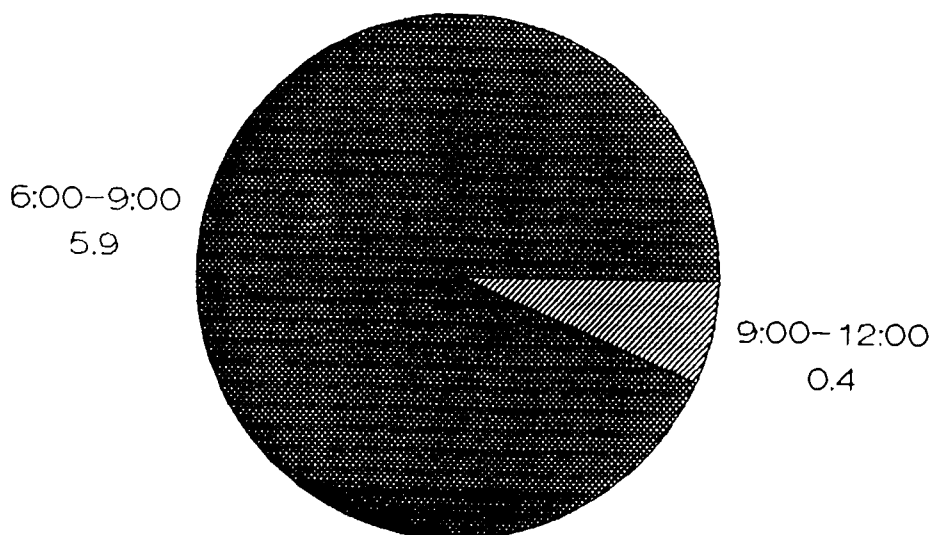


Figure IV.11. Frequency of event 3 (quivers), expressed per hour of total protocol time, per category.

Figure IV.12 shows the number of times (per hour of total protocol time) that territorial males are seen to 'nip' at the algal covering of the substrate (event 4), for each of the five categories. The territorial males usually fed close to their nest.

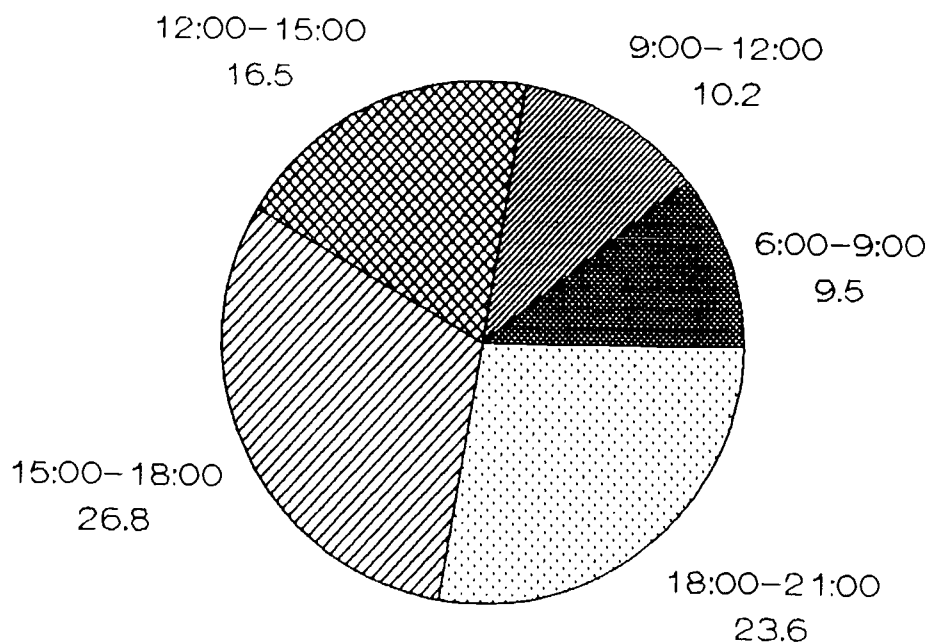


Figure IV.12. Frequency of event 4 (nips the vegetation), expressed per hour of total protocol time, per category.

From Figure IV.12, we see that nipping occurs quite often per hour of total protocol time, for each category. It ranges from 9.5 times during early morning to 26.8 times during late afternoon. Nipping (feeding) is most frequent after midday.

Figure IV.13 displays the number of times (per hour of total protocol time) for each category, that males 'scratch' (scrub) themselves (event 5), by quickly scraping one side of their body against the substrate.

This behaviour is seen in low frequencies during each of the 5 daytime categories. Scratching frequencies vary between 0 and 3.7, per hour; the maximum frequency is recorded during late afternoon.

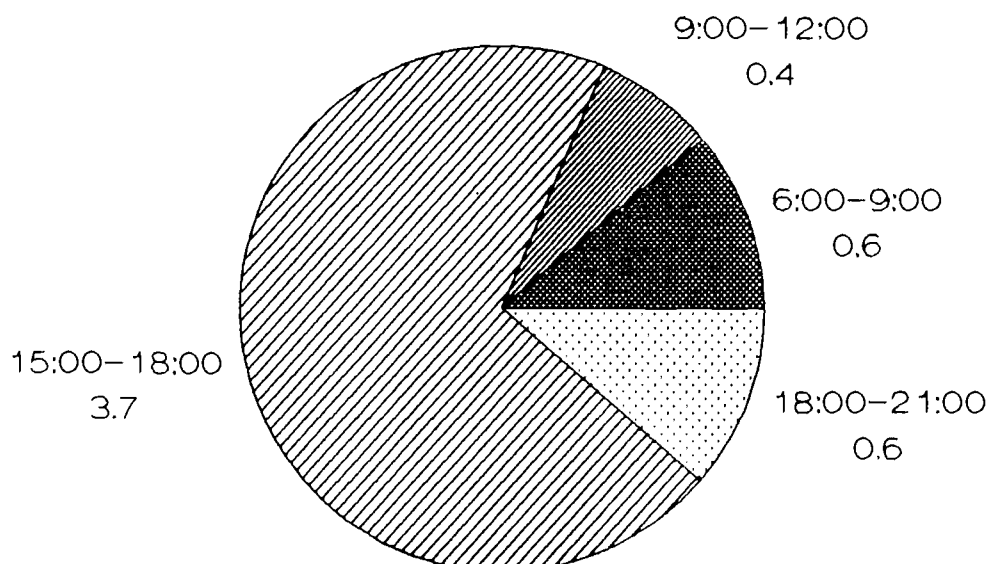


Figure IV.13. Frequency of event 5 (scrapes against the substrate), expressed per hour of total protocol time, per category.

Figure IV.14 shows the number of times that females are seen to enter a nest, as well as the number of times that they are seen to leave a nest (events 6 & 7), expressed per hour of total protocol time for each daytime category.

From the graph, we see that females do not enter a natural hole after 12:00 hours. They are not seen to leave a nest during the afternoon or evening. Both events 6 and 7 (female enters and female leaves the nest) are seen in very low numbers per category. Frequencies of event 6 (females enters) vary between 0 and 0.8, per hour, whilst frequencies of event 7 (female leaves) range from 0 to 1.4 per hour of total protocol time for a daytime category.

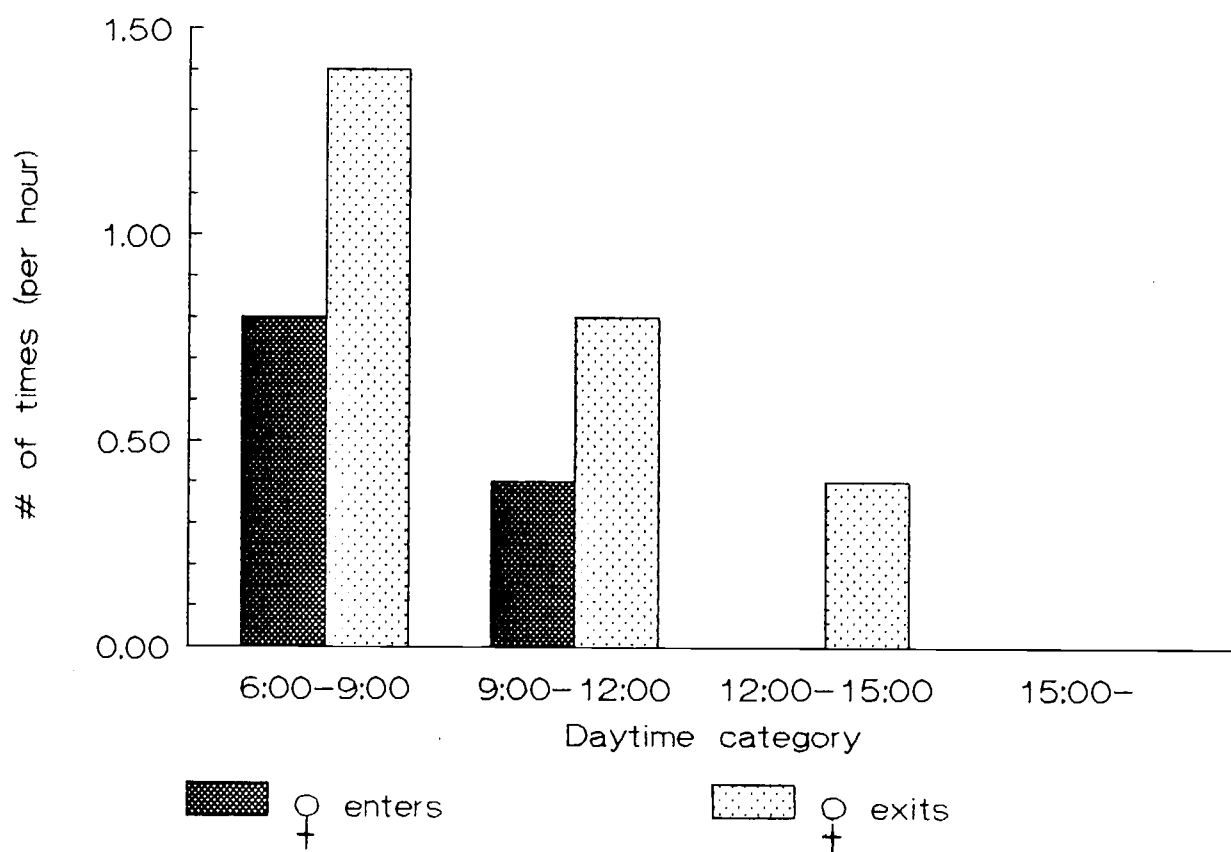


Figure IV.14. Frequency of event 5 and 6 (female enters and female leaves hole), expressed per hour of total protocol time, per category.

* DISCUSSION AND CONCLUSIONS *

I. NATURAL HOLES

I.A. OCCUPATION OF NATURAL HOLES

In this research, *B.incognitus* males are territorial from May to August. Observations on the fourteen marked natural holes show that once *B.incognitus* has 'established' its territory (occupies its hole for more than 2 successive days), it never 'loses its nest' to another male blenny. Holes which are deserted during the reproductive season can be re-used by another male several days later. Most (10 of 17 = ± 59 %) males stop occupying their hole during the month of July. By the beginning of August, all males have deserted their natural hole. Most (14 of 17 = ± 82 %) males have an estimated guarding time of less than 45 days.

To my knowledge, no data has been published on the length of time that male blennies guard their nest. Côte & Hunte (1989), having done research on *B.atlanticus*, a Caribbean *Blennius* species, found that larger males guard longer per reproductive period. These males also spend more time (per day) guarding their nest. Regretfully, the size of the territorial males was not measured during this research period, so that a comparison could not be made between male size and the date on which a male deserted its hole.

The occupation data of the natural holes will be compared with the occupation of the artificial holes, in section II.E of the discussion and conclusions.

TIP

In order to get a more accurate view of the occupation during the course of the season, the natural holes needed to be checked regularly (daily) at approximately the same time during the day.

I.B. ECOLOGICAL ASPECTS OF NATURAL HOLES

In order to conclude that *B.incognitus* shows preference for a certain nest type, size or location, we need to assume that several different holes are available to the territorial males so that males each have a similar choice of nest.

B. incognitus is found in the littoral zone, occupying holes which are surrounded by a low algal vegetation. The substrate in which the nest is situated, is mostly granite (rock). The oscula of 2 species of sponge (*Ircinia muscarum* and *Spongia officinalis*) are also suitable nest sites. Literature indicates that territorial males readily use empty holes of the bivalve *Lithophaga lithophaga*, which are very similar in shape. Cracks and crevices originating from abiotic erosion are also used, but these are much more variable in shape.

In this research, the minimum distance found between two natural holes is 35 cm, although *B. incognitus* males occupying an artificial hole were spaced only ± 10 cm apart. For the 14 natural holes, the mean distance to the nearest neighbour is 1.35 m (s.d. = 1.22). Koppel (1988) studied habitat selection and space partitioning among two species of blennies; she found that, for 23 *B. incognitus* males, the average distance to the nearest neighbour is 72.3 cm (s.d. = 31).

Of the 14 natural holes occupied by *B. incognitus* near Stareso, 10 are situated between 0.5 and 1 m. The deepest hole is situated at 4.8 m. The average depth for the 14 holes is 1.22 m (s.d. = 1.1).

The depth under the water surface, at which a hole is situated, was also measured by Koppel (1988). She found 23 *B. incognitus* nests between depths of 0.2 and 1.5 m, the average depth being 0.86 m (s.d. = 0.28). Illich & Kotrschal (1990) found *B. incognitus* having a broader depth range, from 0 to below 4 m. They found *B. incognitus* to be most abundant between 0.5 and 2 m, decreasing in abundance with increasing depth. This compares with the results of this research.

The 14 natural *B. incognitus* holes around Stareso are situated in sunlit areas, not highly exposed to waves. On the exposure to waves, Illich & Kotrschal (1990) found *B. incognitus* males at greater depths than *B. sphinx* (a typical surge zone species) as well as *B. canevae* (also an inhabitant of a more exposed coast). Louisy (1983) found *B. incognitus* to be photophillic, not inhabiting holes in dark places (caves) or underneath overhanging rocks. The results of this research agree with the above literature data.

In this research, 7 of 12 ($\pm 58\%$) *B. incognitus* males occupy a hole which faces between South and West ($180 - 270^\circ$). Literature data on the orientation of nests occupied by *Blennius* species could not be found.

In this research, the entrance size of 14 different natural holes occupied by *B. incognitus* was measured. The minimum entrance diameter found is 7.3 mm; the maximum entrance diameter found is 42.7 mm. The mean minimum entrance diameter is 11.3 mm (s.d. = 3.21) and the mean maximum entrance diameter is 15.6 mm (s.d. = 9.97).

Kotrschal (1988) measured entrance diameters of one 'standard type' of shelter (only empty holes of endolithic bivalves), which were occupied by different *Blennius* species.

He found that *B.incognitus* occupies shelters with a minimum entrance diameter of 11.5 mm and a maximum entrance diameter of 18.0 mm; the mean of the average (of minimum and maximum) hole diameter, of 40 holes, being 15.1 mm (s.d.= 3.2).

Kotrschal found entrance size to be correlated to male head size.

Koppel (1988) also measured entrances of bore holes occupied by *B.incognitus*. She found an average diameter of 14.57 mm (s.d.= 4.12, n = 21), but no correlation between entrance diameter and male body size.

The hole depth of the 14 natural shelters in this research, varies between 15.4 and 58.7 mm, the average being 32.4 mm (s.d.= 9.70).

Both Koppel (1988) and Kotrschal (1988) measured hole depth of various bore holes occupied by *B.incognitus* males. According to Koppel, *B.incognitus* prefers holes which are ± 12 cm deep. Kotrschal measured the hole depths of 40 holes, and found an average depth of 69.4 mm (s.d.= 15.9).

The average hole depth found in this research, is much lower than the hole depths found by Koppel and Kotrschal. This discrepancy, as well as the difference in minimum and maximum entrance diameter, is probably caused by the different types of hole which were measured; Koppel and Kotrschal only measured occupied bore holes of the mussel *Lithophaga*, which are similar in shape. The hole depths of such 'standard' holes may be larger than hole depths of various other suitable natural holes, more variable in shape and size.

In this research, hole volume varies greatly, ranging from 2 to 20 ml. The average volume for 14 natural holes occupied by *B.incognitus* is 6.25 ml (s.d.= 5.35).

Literature data on the hole volume of shelters occupied by *B.incognitus* could not be found. De Graaf (1983) first used a syringe with a testing liquid to measure hole volume. Of 16 holes occupied by *B.sphinx*, he found hole volume to vary between 2 and 24 ml, the mean volume being 9 ml (s.d.= 6.4).

In this research, most (± 79 %) of the 14 natural holes have an upward slope, their hole entrance lying less deep than the end of the hole.

Koppel (1988) also measured angles of inclination, for 21 holes occupied by *B.incognitus*. In the laboratory she found a tendency for downward sloping of the nest, but in her field research this species did not show preference for a certain angle of inclination. This differs with the results of this research, where *B.incognitus* seems to prefer an upward slope. Of course it may be that such holes are more easily spotted by an observer, who generally looks downwards, compared to holes which have an entrance lying deeper than the hole end.

CONCLUSIONS

B.incognitus males inhabit nests surrounded by a low vegetation of algae, in the shallow littoral zone. This species occupies holes at depths of 0 to 5 m under the water surface, in sun-lit areas not highly exposed to waves.

Territorial males occupy empty holes of mussels, cracks or crevices in rocky substrate, oscula of sponges, as well as glass test-tubes. Distances less than a meter are commonly found between two neighbouring territorial males.

B.incognitus seems to prefer holes with an upward slope, facing South to West. Holes vary very much in size and shape, as is seen in results on entrance diameter, hole depth and hole volume.

TIPS

In this research, only 14 natural holes which were occupied by *B.incognitus* over a period of time, were studied. Preferences for a certain hole size, shape or location may be more clear if ecological parameters on and around many more natural nest sites of this species are measured.

A parameter which may be interesting to look at, is the area of the hole entrance. This may be compared with data on head size or body width of the resident male. Also, the length of the hole may be compared to male body length to get an idea of the 'fit' of a male in its chosen hole.

It would be interesting to see if the length of time in which males occupy a hole is related to ecological parameters of that hole.

Aquarium experiments may also be done, as test-tubes are readily used by territorial *B.incognitus* males. In this way, only one or two aspects (parameters) of artificial holes may be varied whilst other conditions can be kept as similar as possible. Individual males may thus be offered the same choice of nests so that preferences for certain (artificial) holes may be observed.

II. ARTIFICIAL HOLES

II.A. OCCUPATION OF GLASS TEST-TUBES

The glass test-tubes, ± 10 cm apart in concrete blocks, are used by *B.incognitus*, *B.sphinx* and rarely by *B.zvonimiri*. The 2 former species occupy the artificial holes throughout the research period, from May to August.

De Graaf (1983), having done research on *B.sphinx*, was the first to use artificial (glass) holes of different shapes and sizes. He used shelters that were larger than both test-tube sizes used in this research. *B.sphinx*, the main occupant in his experiments, prefers narrow, deep holes (ie. test-tube shape). *B.incognitus* is also occasionally seen in the large test-tubes. To attract *B.sphinx*, he placed 'extra-large' tubes (12 mm diameter, hole depth of 120 mm) at 0.5 m under the water surface, the distance between the tubes being ± 20 cm.

Kraak (1988) used the same sized test-tubes as De Graaf, in her research on female choice (*B.sphinx*). Test-tubes were placed at a depth of 0.6 m, the minimum distance between two tubes being 8 cm.

Louisy (1983) used aquaria with glass test-tubes in polystyrene foam, for his behavioural studies on *B.incognitus* and *B.zvonimiri*, both of which readily use these artificial holes.

CONCLUSION

We may conclude that *B.incognitus* males, as well as *B.sphinx* and *B.zvonimiri* males, readily occupy glass test-tubes as their nest site. Glass artificial holes can thus be a useful tool in studying the biology of these (and possibly other) *Blennius* species. Per species, a 'suitable' tube size and location need to be chosen, to enhance the occupation of the artificial holes by the wanted species.

II.B. OCCUPATION AT DIFFERENT DEPTHS

In this research, blocks I and II, placed at 0.4 and at 0.9 m, are fully occupied during a period of time in June and July. The artificial holes in block IV, at 2.1 m under the water surface, are never occupied by a territorial male blenny throughout the research period.

B.sphinx is only found in test-tubes at depths less than 1 m, whilst *B.incognitus* is seen to occupy artificial holes at 0.4, 0.9, and 1.6 m. *B.zvonimiri* is rarely seen (at 0.9 m) during May. Data on the overall occupation (percentages calculated at the end of the season) of artificial holes, show *B.sphinx* to be the most frequent occupant at 0.4 m (thrice the number of times in which *B.incognitus* is seen at this depth). At 0.9 m, *B.sphinx* and *B.incognitus* occupy holes in approximately the same numbers. At 1.6 m, *B.incognitus* is the only occupant species.

Illich & Kotrschal (1990) published depth distributions of the two most common species found in the artificial holes during this research. They found *B.sphinx* having a depth range of 0 to 1.5 m. This species is most abundant between 0 and 0.5 m under the water surface. *B.incognitus* is found to have a broader depth range, from 0 to 4 m. They found *B.incognitus* to be the most common *Blennius* species at their study site (Figarola Island, Istria, Yugoslavia). This species is most abundant between 0.5 and 2 m; its numbers decrease from 0.5 to 4 m.

CONCLUSION

The differences found in the occupation of artificial holes placed at different depths agree with the literature data on natural depth distributions for *B.incognitus* and *B.sphinx*. Although *B.incognitus* does occur at 2.1 m, it is not seen to occupy artificial holes at this depth; preference may be given to tubes at shallower depths (i.e. blocks I and II, unlike block III, were fully occupied over a period of time during June and July).

II.C. OCCUPATION OF DIFFERENT TEST-TUBE SIZES

The small sized test-tubes used in this research, are 8.5 mm in diameter and have a hole depth of 79 mm. The large tubes are 10 mm in diameter and have a hole depth of 98 mm.

The small and large sized test-tubes, used by *B.incognitus* and *B.sphinx*, are occupied in different ratios. *B.sphinx* males occupy significantly (χ^2 , $p > 5\%$) more large than small sized tubes at 0.4 m, calculated over the whole season. At 0.9 m, differences between the overall occupation of small and large sized tubes by the different species of blenny, are not significant (χ^2 , $p > 5\%$). *B.incognitus* is never seen to occupy a large sized tube at 0.4 m, although both test-tube sizes are used at 0.9 and at 1.6 m. At 0.9 m, this species occupies similar numbers of both sizes of test-tube. At 1.6 m, *B.incognitus* occupies a large sized tube in more than double the number of times in which this species is found in a small sized tube.

Kotrschal (1988), in his research on the utilisation of empty holes of boring mussels by several *Blennius* species, found that hole size is related to body size. Compared to other species of *Blenniidae*, both *B.incognitus* and *B.sphinx* use shelters which are relatively shallow. When disturbed mechanically or chemically, territorial males occupying a 'good-fitting' hole stay in their nest for a longer period of time than males defending a less suitable (too 'loose' or too 'tight') hole. Of the 40 individuals measured, Kotrschal found *B.incognitus* with an average standard length (total body length excluding the tail fin) of 49.9 mm (s.d. = 5.4). Twenty *B.sphinx* males have an average standard length of 54.4 mm (s.d. = 7.1). In Kotrschal's research, these two species use holes with entrances that are correlated to male head size. *B.incognitus* uses bore holes with a mean entrance diameter of 15.1 mm (s.d. = 3.2); *B.sphinx* males use holes with an average diameter of 14.1 mm (s.d. = 3.0).

Koppel (1988) measured 21 bore holes occupied by *B.incognitus* and found these having a mean entrance diameter of 14.57 mm (s.d. = 4.12). As mentioned earlier, she could not detect any correlation between hole diameter and body size.

CONCLUSION

Both the small and large sized test-tubes are used by *B.incognitus* and *B.sphinx*, although these artificial holes generally have narrower entrances than empty holes of bore mussels (shallower and wider than test-tubes) which are commonly used by these species.

In order to study *B.incognitus*, both small and large sized test-tubes can be used to attract territorial males at depths below 1 m. Small tubes would be more suitable at shallower depths, as *B.incognitus* shares this depth range with a larger competitor, *B.sphinx*. For studies on the latter species, the large (or larger sizes of) artificial glass test-tubes are more suitable, at depths of 0 to 1 m.

II.D. OCCUPATION AND WATER TEMPERATURE

As said before, blocks I and II are all fully occupied over a period of time during June and July. *B.sphinx* males start to occupy a hole during May. Numbers of this species do not increase during June, but remain stable until the end of July, after which they decrease. The number of *B.incognitus* males increases during May and the beginning of June, remains stable until the end of July and decreases during August.

Kraak (1988), having done research on *B.sphinx*, suggests that the start of the reproductive season may possibly be related to day length and/or water temperature.

In this research, most territorial *B.sphinx* males start to occupy a hole during May, when the water temperature varies between 14 and 16°C; only a few *B.incognitus* males start to occupy a test-tube during this month. However, many more of the latter species start to occupy an artificial hole during the beginning of June, the water temperature having reached 16°C by the end of May.

CONCLUSION

Literature indicates that the reproductive period of *B.sphinx* starts at the end of April and continues through June and July (Zander in Whitehead *et al.*).

In this research, *B.sphinx* males are still territorial during August. *B.incognitus* is known to have a reproductive period from May to August; this is confirmed by the data on the occupation of the artificial holes. It may be that *B.incognitus* starts to be territorial at higher water temperatures (above 16°C) than *B.sphinx*. For both of these *Blennius* species, highest numbers of territorial males are seen at the blocks during June-July.

II.E. OCCUPATION OF NATURAL AND ARTIFICIAL HOLES

Compared to males guarding a natural nest site (all of which stop guarding their hole by the end of July), *B.incognitus* males which occupy an artificial hole are seen to desert their tube later on during the season, during July and August.

Possible explanations for this difference seen at the end of the season, between males guarding a natural or an artificial hole, may be the following:

A group of *B.incognitus* males might, for different reasons, attract conspecific females more easily than a 'lone' individual. A group of territorial males may be more easily found by a female, due to optic and olfactory signals which are concentrated in a small area; a female may prefer having a wide choice of males within a small area or eggs which are deposited in a 'group hole' may have higher chances of survival than eggs deposited in a 'lone hole'.

Thus, males occupying an artificial hole, with neighbours only ± 10 cm away, may remain territorial for a longer period of time at the end of the reproductive season than a lonely territorial male.

This idea could be tested by comparing the occupation of the artificial holes with the occupation of a group of natural holes, situated close to each other. Desertion dates of lonely males may also be compared with the times at which 'group males' stop guarding their nest.

TIPS

To get a more accurate view of the occupation of artificial holes, each hole needed to be observed regularly. Daily observations, made at the same time each day, would give accurate data on the occupation of tubes during the course of time. In this research, the blocks were not checked very regularly towards the end of the season as well as being checked at different times during the day.

In order to accurately compare data on the occupation of the different blocks, the blocks needed to be identical. In this research, block I had only 4 artificial holes (1 small and 3 large sized tubes) whilst the remaining three blocks were identical, each having 4 small and 4 large sized test-tubes. Care needs to be taken when comparing the (compensated) results on the occupation of block I with results on the other blocks.

Preference for holes at a certain depth by the different *Blennius* species may be more clear, when many more test-tubes are offered and at more depths (smaller depth intervals). In the same way, if more test-tube sizes are used and high numbers of each size are offered, preference for a particular hole size may be more easily observed.

Thus, to be able to conclude that clear preference exists for a certain tube size, tube depth or depth under the water surface, an abundance of artificial holes must be offered.

Preference can only be fairly concluded if each territorial male has been offered the (approximate) same choice of artificial holes. Various different factors, such as food supply, the presence of (or distance to) particular neighbours may influence nest choice; these need to be (made) as similar as possible so that differences in occupation may be attributed to one or two parameters (i.e. tube size, depth under the water surface) only.

III. MORPHOLOGICAL ASPECTS

In this research, the average total length of 12 territorial *B.incognitus* males at the end of August, is 43 mm (s.d.= 4.2).

According to Louisy (1980), *B.incognitus* reaches a maximum total length of 8 cm. Other researchers have measured standard length (total length excluding the length of the tail fin); Kotrschal (1988) found an average standard length of 49.9 mm (s.d.= 5.4) for 40 territorial males, Koppel (1988) found the same mean standard length of 49.9 mm (s.d.= 7.5) for 37 males. The average total length of males in this research is lower than the averages for standard length found by Kotrschal and Koppel.

Kotrschal & Goldschmid (1981), having done research on the population structure of *B.incognitus*, believe that individuals gain sexual maturity after one year; males are then ± 40 mm and females are ± 35 mm in length. After the first year of life, both sexes reach ± 60 % of their possible maximum length. Growth rate then diminishes to 5-6 mm per year. A distinct sexual dimorphism in length is seen; males are 13-15 % longer than females throughout their whole lifespan. *B.incognitus* usually reaches an age of 4 years, 5 years being the maximum longevity. The age of these blennies was determined by studying their otoliths; annuli are laid down annually, the transparent zones being formed during periods of lowest growth rates in early spring.

The average body weight is 0.85 g (s.d.= 0.24) for the 12 territorial males near Stareso. This is much lower than the 2-4 g mentioned by Illich & Kotrschal (1990), in their paper on various *Blennioid* fishes.

In this research, the territorial males were caught and weighed at the end of the reproductive season (late August); the larger males may have stopped guarding earlier on in the season or smaller males may guard longer or become territorial later on in the season. In *B.atlanticus* (Côte and Hunte, 1989), large males guard longer per reproductive period than small males.

In this research, the average gonad weight of 11 territorial males at the end of August is 0.0047 g (s.d.= 0.0014). No data could be found on gonad weights of blennies.

De Jonge & Videler (1989), having studied the reproductive strategies of 2 *Tripterygion* species, published gonad weights of these species. They found that gonads decrease gradually in size towards the end of the spawning season until, in winter, the gonads are at rest.

This may also be true for territorial *B.incognitus* males. Gonad weights measured at the end of the reproductive season may be low, compared to gonad weights during the season (no data was gathered on the latter). Smaller, lighter territorial males have relatively heavier gonads compared to larger, heavier males. Small males may start occupying a hole at a later point in time compared to larger males. Gonads of small males would thus be activated late during the season and may start to diminish in weight later on in the season than the gonads of larger males.

CONCLUSIONS

Compared to literature data, the 12 territorial males near Stareso are small, having low body weights and lengths at the end of August. It is possible that large territorial males stop occupying a hole earlier on in the season. Territorial males were not all measured at the same time during the season, by the various researchers, so that the data may not compare well. Also, the *B.incognitus* population at Stareso could be made up of smaller individuals than populations elsewhere. Kotrschal (1988) and Koppel (1988) measured only *B.incognitus* males which occupied an empty hole of a bore mussel; these are generally larger than the different natural holes measured around Stareso. Thus, differences between literature data and the data of this research of body size and weight of territorial males may be caused by several different factors.

TIPS

In this research, morphological data was obtained from a low number of territorial males at the end of August. Many more territorial males need to be caught and measured to give more accurate data on different morphological parameters. It would be interesting to compare certain morphological aspects of territorial males with their choice of hole; parameters such as head size, body diameter and standard length may be measured for this purpose. The age of the fish can be determined by careful examination of the otoliths; this was not done in this research. Also interesting to look at, is the morphology of individuals during the course of the season. Population samples (of males and females) could be taken at different points during the season, so that various parameters could be measured (preference goes to parameters which can be measured under water, on live fish). Data obtained at different times during the season could then be compared, as well as differences found between territorial males, non-territorial males and females.

IV. BEHAVIOURAL ASPECTS

Non-social behaviours (such as swimming, scratching, feeding, and position at rest) shown by *B.incognitus* are common to many other *Blennius* species. Wirtz (1978) describes several non-social and social behaviours common to *Blenniidae* as well as behaviours displayed by *Tripterygion* species.

B.incognitus males are not very aggressive towards intruders. Agonistic reactions of different degrees are mostly shown to conspecific males and in a much lesser extent to females or individuals of another species. Interspecific interaction is mostly seen between a territorial male and another blenny, or occasionally a *Tripterygion*. Males usually retreat inside their nest on the approach of larger species of fish.

Courtship is more specific per *Blennius* species. The following courting behaviour of *B.incognitus* was described by Abel (1964):

The male starts to display courting behaviour when a female comes within 20-30 cm of the nest. On the arrival of a female, the male rears up the anterior part of its body and, in doing so, nearly leaves the nest. The male repeatedly rears up and frequently trembles (quivers) its body. The female, with open mouth, presses her light coloured abdomen forwards. The male may repeatedly swim in and out of the hole, showing the position of the nest. The male may occasionally pretend to attack the female. The female enters the nest quickly, and the male waits motionless in front of the hole whilst the female deposits her eggs. Occasionally the male successfully enters the nest, tail first, joining the female. The time of deposition, related to the size of the female, varies between 10 and 60 minutes.

Throughout this research, *B.incognitus* is not seen to exactly or fully complete the sequence as described by Abel. Courtship behaviour lasts only a very short time and females are often inside a hole within a minute of their arrival. Males sometimes start displaying courtship behaviour when a female has come within 20 cm of the nest. Occasionally, males completely ignore a female, even when she waits next to the hole entrance. These observations have also been described by Videler (1980).

Rearing up and trembling is frequently seen, but the female's response is not as clear as Abel's description. Females were rarely 'attacked' by the male, although they are sometimes nipped at. Throughout this research, males rarely swam in and out of their nest, showing the position of the hole entrance. They often swim out of their nest, hopping over or against a waiting female. The female usually enters the hole quickly, but does not always remain inside to deposit eggs. Territorial males were always seen to enter (or attempt to enter) the nest, whilst a female was inside. Usually, this succeeded and the male was seen to quiver once in a while (probably fertilising the eggs). Females leaving the nest were often nipped at or chased away by the male; this behaviour is also mentioned by Videler (1980).

Parental care, as in other endemic *Blennius* species, is done by the male. *B.incognitus* males ventilate their nest by waving their lateral fins up and down, in a similar fashion to that shown by *B.sphinx* males, described by De Graaf (1983).

B.incognitus is active during the light period, like other species of *Blenniidae* (Wirtz, 1978). This species spends most of the day at rest. Territorial males also spend a large part of the day totally inside their hole. Also, a large amount of time is spent on regularly ventilating the haptic hole. Small amounts of time are spent on swimming, agonistic and courtship display, as well as on intraspecific and interspecific interactions. Territorial males are frequently seen to change their guarding position, throughout the day.

Data on daily activity of *B.incognitus* could not be found. Literature was found on *B.atlanticus*; like *B.incognitus*, territorial males of this Caribbean species guard a nest (crevice or hole with one or more entrances) in a surrounding territory. Unlike *B.incognitus*, *B.atlanticus* spawns during the first 3 hours of daylight only, all year round, around full moon (Côte & Hunte, 1989). Male-male interactions are rarely seen during the morning, and only occur between neighbouring territorial males during the afternoon (Nursall, 1977).

Nursall (1981) looked at the activity budget of *B.atlanticus*, and found this species to be diurnal, like *B.incognitus*. Territorial males spend only 5 % of their daily activity budget in their crevice (hole); 60 % is spent resting on the substrate, overlooking their territory. Territorial males of this species spent 15 % swimming, 8.5 % feeding (concentrated in the afternoon) and 2.5 % interacting with other individuals. In the remaining 9 % of time, Nursall records a male as out of site.

CONCLUSION

Resting, ventilating the hole, being totally inside the nest and interspecific interaction are seen in similar amounts of time, during the 5 daytime categories (spanning 3 hours each, from 06:00 to 21:00). Swimming, feeding, scratching, and leaving the nest are mostly seen during the afternoon, whilst courtship behaviour, trembling and spawning occurs most during morning hours.

The daily activity budget of *B.incognitus* differs greatly from that of *B.atlanticus*.

TIPS

To be able to describe different behaviours in more detail, a video-recorder could be used to record different activities on film, which can then be carefully analyzed.

To allow for more (different) behaviours to be registered in a protocol, the protocol duration needs to be longer than 10 minutes per male.

It would have been better to divide state 2 (resting) into two states; namely resting (in between swimming) outside of the hole and resting (from fanning) partly inside the nest. Resting totally inside the nest was recorded under state 6 (inside nest).

Not only the time spent on ventilating the hole, but also the frequency of fanning influences the water circulation around the eggs. The ventilation of the nest could be looked at more closely and the movements of the lateral fins could also be registered, to get an idea of ventilation frequency.

A video-recorder could be 'permanently' situated in front of a nest, recording behaviour in between observations made by an observer. Of course this is only possible in areas where water turbulence is at a minimum. This would give a more complete picture of the daily activity of one specific male.

It would be interesting to see if there are differences in daily activity between individual males. Also, activity budgets may change during the season. In order to investigate such differences, a large number of protocols, of many different males, need to be made. Regretfully, the event-recorder was only used during July and August and only a few males still occupied a nest long enough to be frequently 'protocolled' at that time.

Also, it would be interesting to compare daily activity between males guarding an artificial nest and those guarding a natural nest. Males guarding a test-tube possibly spend more time interacting with their close by neighbours than 'lone' males which occupy a natural hole. Also, feeding in the direct vicinity of the test-tubes was not possible (due to the low algal covering on the concrete blocks), so that males guarding a test-tube may spend more time to feed elsewhere. Thus, there are various possibilities when studying behaviour.

Several ecological, morphological and behavioural aspects of territorial males of *B.incognitus* have been studied at Stareso. Methods used in this research project leave room for improvement as well as several possibilities for further research on the biology of this *Blennius* species.

This paper contributes to the knowledge on *B.incognitus* and may be used in later comparative studies concerning the speciation of the *Blenniidae* in the Mediterranean.

* * * * *

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Appendix I.a. The occupation of the 14 marked natural nest sites during the course of time.

		Male												
Date	Day #	b7	b2	b1	b4	b3	b5	b6	b8	b9	b10	b13	b14	b11
22-4	0													
23-4	1	1	1											
24-4	2	1	1											
25-5	3	1	1											
26-5	4	1	1											
:	:													
29-4	7	1	1											
:	:													
02-5	10	1	1											
03-5	11	1	1											
:	:													
06-5	14	1	1											
07-5	15	1	1											
08-5	16													
09-5	17	1	1											
10-5	18	1	1											
:	:													
13-5	21	1	1											
:	:													
17-5	25		sx											
:	:													
:	:		b2'	b1	b4									
24-5	32	1	1	1	1									
:	:													
27-5	35	0	1	1	1									
28-5	36		1	1	1									
29-5	37													
30-5	38		1	1	1									
:	:													
02-6	41		1	1	1									
03-6	42		1											
04-6	43		1	1	1	b3	b5	b6						
05-6	44		1	1	1	1	1	1						
06-6	45		1	1	0	1	1	1						
07-6	46		1	1	0	1	1	1						
08-6	47		1	1	1	sx	1	1						
09-6	48								b8	b9	b10			
10-6	49		1	1	0	0	1	1	1	1	1			
11-6	50		1	1		0	1	1	1	1	1			
12-6	51		1											
13-6	52													
14-6	53		1											
:	:					b3'								
17-6	56		1	1		1	1	1	0	1	1			
18-6	57		1	1		1	1	1	1	1	1			
19-6	58		1											
20-6	59		1											

continued...

APPENDIX - I.a.

Date	Day #	Male											
		b7	b2	b1	b4	b3	b5	b6	b8	b9	b10	b13	b14
21-6	60		1										
22-6	61												
23-6	62				b4'								
24-6	63		1	1	1	1	1	1	1	1	1		b14
25-6	64		1	1	1	1	1	1	1	1	1	b13	1
26-6	65		1	1	1	1	1	1	0	1	1	1	1
:	:												
01-7	70		1										
02-7	71		1	1	0	1	1	1	1	1	1	1	
03-7	72		1										
04-7	73		1	1	1	1	1	1	1	1	1	1	b11
05-7	74		1					1	1				1
:	:												
08-7	77		1		1			1	1				1
:	:												
11-7	80		1	1	1			1	1				1
12-7	81		1					1	1				1
:	:												
15-7	84		1	1	1	1	0	1	1	1	1	1	1
16-7	85		1	1	1	1		1	0				1
17-7	86		1	1	1	1		1					1
18-7	87		0	1	0	sx		1					1
19-7	88			1	1	0		1		1	1	1	1
20-7	89			1	1			1					1
:	:												
23-7	92			1				1		1			1
24-7	93			1	1			1					1
25-7	94		b2"										
26-7	95		1					1					1
:	:												
05-8	105		1	0	0			1		0	0	0	1
06-8	106		1					1					1
07-8	107												
08-8	108		1					1					1
09-8	109		0					1					0
:	:												
13-8	113							0					

Key: 1 = B.inc. present
0 = B.inc. absent
sx = B.sx. present

Appendix I.b. Summarised data on the occupation of 14 marked natural nest sites: first and last day a male was seen to occupy its hole, as well as the total number of observations made per male and the estimated number of guarding days.

MALE	1st day	last day	# of obs.	'guarding days' #
b3	44	46	3	3
b2"	95	108	4	14
b4	32	47	8	16
b2	1	21	12	21
b13	65	88	5	24
b14	64	92	7	24
b5	44	73	13	30
b3'	56	86	10	31
b4'	63	93	12	31
b7	1	32	13	32
b8	49	84	12	36
b11	73	108	17	36
b10	49	88	11	40
b9	49	92	12	44
b2'	32	86	34	55
b1	32	93	28	62
b6	44	109	30	66
Average =				33.2
s.d. =				16.2
n =				17

Appendix I.c. Data on the different parameters measured on and around the 14 marked natural nest sites.

Hole	Substrate - type	Distance (m) to nearest neighbour	Depth (m)	Compass direction (bearing)
n 1	sponge - <i>I.muscarum</i>	0.35 (n 3)	1.1	260
n 2	tetrapod - concrete	2.70 (n 11)	2.1	180
n 3	sponge - <i>I.muscarum</i>	0.35 (n 1)	0.9	250
n 4 *	sponge - <i>I.muscarum</i>	1.50 (n 3)	0.9	220
n 5	rock - granite	0.60 (n 14)	0.6	170
n 6	sponge - <i>I.muscarum</i>	1.50 (n 8)	0.9	270
n 7	rock - granite	4.80 (n 11)	4.8	70
n 8 *	sponge - <i>I.muscarum</i>	0.40 (n 12)	0.8	270
n 9	rock - granite	1.00 (n 10)	0.6	100
n 10	rock - granite	1.00 (n 9)	0.5	60
n 11	tetrapod - concrete	2.70 (n 2)	2.1	180
n 12	sponge - <i>S.officinalis</i>	0.40 (n 8)	0.6	330
n 13	rock - granite	1.10 (n 14)	0.6	
n 14	rock - granite	0.60 (n 5)	0.6	
Average		1.35	1.2	197
s.d		1.22	1.1	82
n		14	14	12

* = 2 entrances

continued...

APPENDIX - I.c.

Hole	Minimum diameter (cm)	Maximum diameter (cm)	Hole depth (cm)	Hole volume (ml)	Angle of inclination (degrees)
n 1	1.61	1.67	2.84	7	-65
n 2	0.81	0.94	3.13	4.5	20
n 3	0.77	1.08	3.24	3.5	65
n 4 *	1.12	1.12	3.31	3	-50
n 5	1.16	3.33	2.83	3	15
n 6	1.05	1.18	3.69	3.5	30
n 7	0.73	0.73	1.54	5	15
n 8 *	1.04	1.08	5.87	12.5	55
n 9	1.68	4.27	3.92	15	45
n 10	1.4	1.4	2.42	3.5	10
n 11	0.82	0.94	3.13	3	10
n 12	0.99	0.99	2.55	2	-80
n 13	1.66	2.22	4.2	20	75
n 14	0.94	0.94	2.71	2	80
	1.13	1.56	3.24	6.3	16
	0.32	0.99	0.97	5.4	48
	14	14	14	14	14

Appendix II.a. The occupation of the 28 artificial holes,
during the course of the research period.

Size Tube	Block I				Block II								Block III								Block IV			
	S 1	L 2	L 3	L 4	S 1	S 3	S 6	S 8	L 2	L 4	L 5	L 7	S 1	S 3	S 6	S 8	L 2	L 4	L 5	L 7	each tube			
Date Day #																								
06-5 14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
07-5 15	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0			
08-5 16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
09-5 17	0	0	0	0	0	SX	0	1	SX	0	0	0	0	0	0	0	0	0	0	1	0			
10-5 18	0	0	SX	0	SX	0	0	0	SX	0	0	0	0	0	0	0	0	0	0	1	0			
11-5 19	0	0	0	SX	SX	1	1	0	SX	0	0	0	0	0	0	0	0	0	0	1	0			
12-5 20																								
13-5 21	0	0	0	SX	SX	1	1	0	SX	SX	0	0	0	0	0	0	0	0	0	1	0			
14-5 22	0	0	SX	0	SX	1	1	SX	SX	SX	0	ZVO	1	0	0	0	0	1	0	1	0			
15-5 23	SX	0	SX	SX	SX	1	1	SX	SX	SX	0	ZVO	1	0	0	0	0	0	0	1	0			
16-5 24	SX	0	SX	SX	SX	1	1	SX	SX	SX	0	ZVO	0	0	0	0	0	0	0	1	0			
17-5 25	0	0	SX	SX	SX	1	1	SX	1	SX	0	ZVO	0	0	0	0	0	0	0	1	0			
18-5 26	0	SX	SX	SX	SX	1	1	SX	1	SX	0	ZVO	0	0	0	0	0	1	0	1	0			
19-5 27																								
20-5 28	SX	SX	SX	SX	SX	1	1	SX	SX	1	0	ZVO	0	0	0	0	0	0	0	1	0			
21-5 29	0	SX	SX	SX	SX	1	1	SX	SX	0	1	ZVO	0	0	0	0	1	0	0	1	0			
22-5 30	0	SX	SX	SX	SX	1	1	SX	SX	SX	1	ZVO	0	0	0	0	0	0	0	1	0			
23-5 31																								
24-5 32	0	SX	SX	SX	SX	ZVO	1	SX	SX	SX	1	1	0	0	0	0	0	0	0	1	0			
;																								
27-5 35	0	SX	SX	SX	SX	ZVO	1	SX	SX	SX	1	1	0	0	0	0	0	0	0	1	0			
28-5 36	0	SX	SX	SX	SX	ZVO	1	SX	SX	SX	1	1	0	0	0	0	0	0	0	1	0			
;																								
04-6 43	0	SX	SX	SX	SX	1	1	SX	SX	SX	1	1	1	1	0	1	0	1	0	1	0			
05-6 44	SX	SX	SX	SX	SX	1	1	SX	SX	SX	1	1	1	0	0	1	1	1	0	1	0			
06-6 45	1	SX	SX	SX	SX	1	1	SX	SX	SX	1	1	1	0	0	1	1	0	1	1	0			
07-6 46																								
08-6 47	1	SX	SX	SX	SX	1	1	SX	SX	SX	1	1	1	0	0	1	0	1	0	1	0			
09-6 48																								
10-6 49	1	SX	SX	SX	SX	1	1	SX	SX	SX	1	1	1	1	0	1	1	1	0	1	0			
11-6 50	1	SX	SX	SX	SX	1	1	SX	SX	SX	1	1	1	1	0	1	1	1	0	1	0			
12-6 51	1	SX	SX	SX	SX	1	1	SX	SX	SX	1	1	1	1	0	1	1	1	0	1	0			

APPENDIX - II.a.

Size Tube	Date Day #	Block I				Block II				Block III										Block IV			
		S 1	L 2	L 3	L 4	S 1	S 3	S 6	S 8	L 2	L 4	L 5	L 7	S 1	S 3	S 6	S 8	L 2	L 4	L 5	L 7	each tube	
17-6 18-6 24-6 02-7 03-7 04-7 15-7 19-7 23-7 05-8 08-8 09-8 13-8 16-8 19-8 20-8 26-8 27-8 28-8	56 57 63 71 72 73 84 88 92 105 108 109 113 116 119 120 126 127 128	1 1 1 1 1 1 1 1 1 1 SX SX SX SX SX SX SX 0 0 0	SX SX SX SX SX SX SX SX SX SX SX SX SX SX SX SX SX SX SX	SX SX SX SX SX SX SX SX SX SX SX SX SX SX SX SX SX SX SX	SX 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	SX SX SX SX SX SX SX SX SX SX SX SX SX SX SX SX SX SX	SX 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	SX 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	SX 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	SX 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0			

0 = tube unoccupied
1 = B.incognitus occupies tube
sx = B.sphinx occupies tube
zvo = B.zvonimir occupies tube

Appendix II.b. Data on the overall occupation of the artificial holes, summarised over all the observations made during the research period.

BLOCK I	# inc	# sx	# zvo	# unocc	# tot
S (1)	14	12	0	16	42
total	14	12	0	16	42
compensated total	56	48	0	64	168
L (2)	0	29	0	13	42
L (3)	0	30	0	12	42
L (4)	0	26	0	16	42
total	0	85	0	41	126
compensated total	0	113	0	55	168
I-Total	14	97	0	57	168
compensated Total	56	161	0	119	336

BLOCK II	# inc	# sx	# zvo	# unocc	# tot
S (1)	8	28	0	6	42
S (3)	25	1	3	13	42
S (6)	28	0	0	14	42
S (8)	3	24	0	15	42
total	64	53	3	48	168
L (2)	3	36	0	3	42
L (4)	1	35	0	6	42
L (5)	27	0	0	15	42
L (7)	25	0	8	9	42
total	56	71	8	33	168
II-TOTAL	120	124	11	81	336

BLOCK III	# inc	# sx	# zvo	# unocc	# tot
S (1)	17	0	0	25	42
S (3)	9	0	0	33	42
S (6)	1	0	0	41	42
S (8)	11	0	0	31	42
total	38	0	0	130	168
L (2)	23	0	0	19	42
L (4)	15	0	0	27	42
L (5)	17	0	0	25	42
L (7)	26	0	0	16	42
total	81	0	0	87	168
III-TOTAL	119	0	0	217	336

Appendix II.c. Levels of occupation (expressed in numbers and in percentages) of the artificial holes during the research period.

		ALL OFFERED TEST-TUBES (28 in total)			
Date	Day #	% TOT	% INC	% SX	% ZVO
07-5	15	3.5	3.6	0	0
08-5	16	7.1	7.1	0	0
09-5	17	14.3	7.1	7.1	0
10-5	18	14.3	3.6	10.7	0
11-5	19	21.4	10.7	10.7	0
13-5	21	25	10.7	14.3	0
14-5	22	39.3	17.9	17.9	3.6
15-5	23	42.9	14.3	25	3.6
16-5	24	39.3	10.7	25	3.6
17-5	25	35.7	14.3	17.9	3.6
18-5	26	42.9	17.9	21.4	3.6
20-5	28	42.9	14.3	25	3.6
21-5	29	42.9	17.9	21.4	3.6
22-5	30	42.9	14.3	25	3.6
24-5	32	42.9	14.3	25	3.6
27-5	35	42.9	14.3	25	3.6
28-5	36	42.9	14.3	25	3.6
04-6	43	57.1	32.1	25	0
05-6	44	60.7	32.1	28.6	0
06-6	45	60.7	35.7	25	0
08-6	47	57.1	32.1	25	0
10-6	49	64.3	39.3	25	0
11-6	50	64.3	39.3	25	0
12-6	51	64.3	39.3	25	0
17-6	56	67.9	42.9	25	0
18-6	57	64.3	39.3	25	0
24-6	63	60.7	35.7	25	0
02-7	71	64.3	39.3	25	0
04-7	73	64.3	39.3	25	0
15-7	84	53.6	32.1	21.4	0
19-7	88	53.6	35.7	17.9	0
23-7	92	50	32.1	17.9	0
05-8	105	32.1	21.4	10.7	0
08-8	108	35.7	17.9	17.9	0
09-8	109	35.7	17.9	17.9	0
13-8	113	35.7	17.9	17.9	0
16-8	116	32.1	17.9	14.3	0
19-8	119	32.1	17.9	14.3	0
20-8	120	28.6	14.3	14.3	0
26-8	126	17.9	7.1	10.7	0
27-8	127	21.4	10.7	10.7	0
28-8	128	14.3	7.1	7.1	0

Appendix II.d. Levels of occupation (number of times a hole was found occupied) of small and large sized test-tubes, per depth under the water surface.

		BLOCK I (n=4)	BLOCK II (n=8)	BLOCK III (n=8)	BLOCK IV (n=8)
Date	Day #	% tot	% tot	% tot	% tot
07-5	15	0	0	12.5	0
08-5	16	0	12.5	12.5	0
09-5	17	0	37.5	12.5	0
10-5	18	25	25	12.5	0
11-5	19	25	50	12.5	0
13-5	21	25	62.5	12.5	0
14-5	22	25	87.5	37.5	0
15-5	23	75	87.5	25	0
16-5	24	75	87.5	12.5	0
17-5	25	50	87.5	12.5	0
18-5	26	75	87.5	25	0
20-5	28	100	87.5	12.5	0
21-5	29	75	87.5	25	0
22-5	30	75	100	12.5	0
24-5	32	75	100	12.5	0
27-5	35	75	100	12.5	0
28-5	36	75	100	12.5	0
04-6	43	75	100	62.5	0
05-6	44	100	100	62.5	0
06-6	45	100	100	62.5	0
08-6	47	100	100	50	0
10-6	49	100	100	75	0
11-6	50	100	100	75	0
12-6	51	100	100	75	0
17-6	56	100	100	87.5	0
18-6	57	100	100	75	0
24-6	63	100	100	62.5	0
02-7	71	100	100	75	0
04-7	73	100	100	75	0
15-7	84	100	100	37.5	0
19-7	88	100	100	37.5	0
23-7	92	75	87.5	50	0
05-8	105	75	50	25	0
08-8	108	75	62.5	25	0
09-8	109	75	62.5	25	0
13-8	113	75	62.5	25	0
16-8	116	50	62.5	25	0
19-8	119	50	62.5	25	0
20-8	120	50	50	25	0
26-8	126	25	25	25	0
27-8	127	25	37.5	25	0
28-8	128	0	25	25	0

Appendix II.e. Level of occupation (number of time a hole was found occupied) of the 13 small sized test-tubes, during the research period.

		SMALL SIZED TEST-TUBES (13 in total)			
Date	Day #	% tot	% Inc	% Sx	% Zvo
07-5	15	0	0	0	0
08-5	16	15.4	15.4	0	0
09-5	17	15.4	7.7	7.7	0
10-5	18	7.7	0	7.7	0
11-5	19	23.1	15.4	7.7	0
13-5	21	23.1	15.4	7.7	0
14-5	22	38.5	23.1	15.4	0
15-5	23	46.2	23.1	23.1	0
16-5	24	38.5	15.4	23.1	0
17-5	25	30.8	15.4	15.4	0
18-5	26	30.8	15.4	15.4	0
20-5	28	38.5	15.4	23.1	0
21-5	29	30.8	15.4	15.4	0
22-5	30	30.8	15.4	15.4	0
24-5	32	30.8	7.7	15.4	7.7
27-5	35	30.8	7.7	15.4	7.7
28-5	36	30.8	7.7	15.4	7.7
04-6	43	53.8	38.5	15.4	0
05-6	44	53.8	30.8	23.1	0
06-6	45	53.8	38.5	15.4	0
08-6	47	53.8	38.5	15.4	0
10-6	49	61.5	46.2	15.4	0
11-6	50	61.5	46.2	15.4	0
12-6	51	61.5	46.2	15.4	0
17-6	56	61.5	46.2	15.4	0
18-6	57	61.5	46.2	15.4	0
24-6	63	53.8	38.5	15.4	0
02-7	71	61.5	46.2	15.4	0
04-7	73	61.5	46.2	15.4	0
15-7	84	46.2	38.5	7.7	0
19-7	88	46.2	38.5	7.7	0
23-7	92	38.5	30.8	7.7	0
05-8	105	15.4	15.4	0	0
08-8	108	15.4	7.7	7.7	0
09-8	109	15.4	7.7	7.7	0
13-8	113	15.4	7.7	7.7	0
16-8	116	15.4	7.7	7.7	0
19-8	119	15.4	7.7	7.7	0
20-8	120	15.4	7.7	7.7	0
26-8	126	7.7	0	7.7	0
27-8	127	7.7	0	7.7	0
28-8	128	0	0	0	0

Appendix II.f. Level of occupation (number of time a hole was found occupied) of the 15 large sized test-tubes, during the research period.

		LARGE SIZED TEST-TUBES (15 in total)			
Date	Day #	% tot	% Inc	% Sx	% Zvo
07-5	15	6.7	6.7	0	0
08-5	16	0	0	0	0
09-5	17	13.3	6.7	6.7	0
10-5	18	20	6.7	13.3	0
11-5	19	20	6.7	13.3	0
13-5	21	26.7	6.7	20	0
14-5	22	40	13.3	20	6.7
15-5	23	40	6.7	26.7	6.7
16-5	24	40	6.7	26.7	6.7
17-5	25	40	13.3	20	6.7
18-5	26	53.3	20	26.7	6.7
20-5	28	46.7	13.3	26.7	6.7
21-5	29	53.3	20	26.7	6.7
22-5	30	53.3	13.3	33.3	6.7
24-5	32	53.3	20	33.3	0
27-5	35	53.3	20	33.3	0
28-5	36	53.3	20	33.3	0
04-6	43	60	26.7	33.3	0
05-6	44	66.7	33.3	33.3	0
06-6	45	66.7	33.3	33.3	0
08-6	47	60	26.7	33.3	0
10-6	49	66.7	33.3	33.3	0
11-6	50	66.7	33.3	33.3	0
12-6	51	66.7	33.3	33.3	0
17-6	56	73.3	40	33.3	0
18-6	57	66.7	33.3	33.3	0
24-6	63	66.7	33.3	33.3	0
02-7	71	66.7	33.3	33.3	0
04-7	73	66.7	33.3	33.3	0
15-7	84	60	26.7	33.3	0
19-7	88	60	33.3	26.7	0
23-7	92	60	33.3	26.7	0
05-8	105	46.7	26.7	20	0
08-8	108	53.3	26.7	26.7	0
09-8	109	53.3	26.7	26.7	0
13-8	113	53.3	26.7	26.7	0
16-8	116	46.7	26.7	20	0
19-8	119	46.7	26.7	20	0
20-8	120	40	20	20	0
26-8	126	26.7	13.3	13.3	0
27-8	127	33.3	20	13.3	0
28-8	128	26.7	13.3	13.3	0

Appendix II.g. Water temperature (in degrees celsius),
measured at a depth of 2 m in the port of Stareso,
between April 22 (day 0) and August 17 (day 117).

Date	Day #	Day	Water temp. (°C)
22-4	0	Mon	14
23-4	1	Tue	14
24-4	2	Wed	14
25-4	3	Thu	14
26-4	4	Fri	14
27-4	5	Sat	14
28-4	6	Sun	14
29-4	7	Mon	14
30-4	8	Tue	14
01-5	9	Wed	14
02-5	10	Thu	14.2
03-5	11	Fri	14.5
04-5	12	Sat	14.5
05-5	13	Sun	14.2
06-5	14	Mon	14.2
07-5	15	Tue	14.3
08-5	16	Wed	14.5
09-5	17	Thu	14.6
10-5	18	Fri	14.6
11-5	19	Sat	14.8
12-5	20	Sun	14.7
13-5	21	Mon	14.6
14-5	22	Tue	14.6
15-5	23	Wed	15.1
16-5	24	Thu	15.5
17-5	25	Fri	15.5
18-5	26	Sat	15.5
19-5	27	Sun	15.6
20-5	28	Mon	15.7
21-5	29	Tue	15.7
22-5	30	Wed	16
23-5	31	Thu	16
24-5	32	Fri	16.5
25-5	33	Sat	16.6
26-5	34	Sun	16.7
27-5	35	Mon	16.8
28-5	36	Tue	17
29-5	37	Wed	17.1
30-5	38	Thu	17.2
31-5	39	Fri	17.4
01-6	40	Sat	17.5
02-6	41	Sun	17.7
03-6	42	Mon	17.8
04-6	43	Tue	17.8
05-6	44	Wed	17.5
06-6	45	Thu	17.5
07-6	46	Fri	17.5
08-6	47	Sat	17.7
09-6	48	Sun	17.9
10-6	49	Mon	18.1
11-6	50	Tue	18.2
12-6	51	Wed	18.4
13-6	52	Thu	18.8
14-6	53	Fri	19
15-6	54	Sat	19.3
16-6	55	Sun	19.7
17-6	56	Mon	20
18-6	57	Tue	19.7
19-6	58	Wed	19
20-6	59	Thu	19

Date	Day #	Day	Water temp. (°C)
21-6	60	Fri	19
22-6	61	Sat	19
23-6	62	Sun	19.2
24-6	63	Mon	19.4
25-6	64	Tue	20
26-6	65	Wed	20.4
27-6	66	Thu	20.4
28-6	67	Fri	20.4
29-6	68	Sat	20.6
30-6	69	Sun	20.7
01-7	70	Mon	20.8
02-7	71	Tue	21
03-7	72	Wed	21
04-7	73	Thu	21.7
05-7	74	Fri	21.9
06-7	75	Sat	22.1
07-7	76	Sun	22.3
08-7	77	Mon	22.5
09-7	78	Tue	22.5
10-7	79	Wed	22.6
11-7	80	Thu	23
12-7	81	Fri	23.4
13-7	82	Sat	23.8
14-7	83	Sun	23.5
15-7	84	Mon	23.7
16-7	85	Tue	23.7
17-7	86	Wed	23.5
18-7	87	Thu	23.6
19-7	88	Fri	23.8
20-7	89	Sat	23.5
21-7	90	Sun	24
22-7	91	Mon	24.5
23-7	92	Tue	24.9
24-7	93	Wed	25
25-7	94	Thu	24.6
26-7	95	Fri	24.1
27-7	96	Sat	24.1
28-7	97	Sun	24.1
29-7	98	Mon	23.8
30-7	99	Tue	24.3
31-7	100	Wed	24.5
01-8	101	Thu	24
02-8	102	Fri	23.4
03-8	103	Sat	23.5
04-8	104	Sun	23.9
05-8	105	Mon	24.2
06-8	106	Tue	24.3
07-8	107	Wed	24.6
08-8	108	Thu	24.8
09-8	109	Fri	25
10-8	110	Sat	24.8
11-8	111	Sun	24.7
12-8	112	Mon	24.6
13-8	113	Tue	24.4
14-8	114	Wed	24.7
15-8	115	Thu	25
16-8	116	Fri	25.5
17-8	117	Sat	26

Appendix III.a. Morphological data of 12 territorial
B.incognitus males, obtained at the end of August.

Male	Total length (cm)	Body weight (g)	Gonad weight (g)	Relative gonad weight (%)
b12	3.7	0.4967	0.0055	1.11
H5	3.8	0.5844	0.0051	0.87
H3	3.8	0.5548	0.0035	0.63
H1	4	0.698	0.0036	0.52
II.1	4.1	0.7567	0.0044	0.58
H6	4.4	0.9927	0.0074	0.75
b2''	4.4	0.8134	0.005	0.61
III.2	4.5	0.8857	0.0036	0.41
II.5	4.7	1.0518	0.0073	0.69
III.5	4.7	0.944	0.0036	0.38
H4	4.8	1.2679	0.0032	0.25
H2	5	1.1053	worms	0
Average	4.3	0.846	0.0047	0.62
s.d	0.4	0.228	0.0014	0.23
n	12	12	11	11

Appendix IV.a. List of protocols made during the research period, divided into five daytime categories of 3 hours each; protocol starting time and true duration (without start and end states) are included.

Protocol starting time		Date	Male	'True protocol time' (-start & -end state) min sec = seconds		
"EARLY MORNING"				total = 12822.7		
6	11	17-7	b2	10	40.2	640.2
6	15	19-7	b11	15	52.1	952.1
6	25	17-7	b11	24	45.5	1485.5
6	43	19-7	b1	16	25.2	985.2
6	58	17-7	b6	10	56.3	656.3
7	0	19-7'	b4	9	39.9	579.9
7	15	17-7	b4	12	0.9	720.9
7	18	19-7	b6	7	47.8	467.8
7	30	17-7	b3	15	14.1	914.1
7	47	17-7	b1	9	48.4	588.4
8	15	05-7	b2	29	0.3	1740.3
8	50	05-7	b11	51	32	3092
"LATE MORNING"				total = 8856.2		
9	39	18-7	b11	9	25.4	565.4
9	40	08-7	b2	11	40.6	700.6
9	55	08-7	b11	10	1.1	601.1
10	1	18-7	b6	9	16.5	556.5
10	12	08-7	b6	6	54.3	414.3
10	25	08-7	b8	8	53.8	533.8
10	25	12-7	b2	10	1.8	601.8
10	38	08-7	b4	10	8.9	608.9
10	38	12-7	b11	9	48.9	588.9
10	56	12-7	b6	9	33.5	573.5
11	13	20-7	b11	4	29.6	269.6
11	15	12-7	b8	8	52.8	532.8
11	38	20-7	b6	7	56.2	476.2
11	45	11-7	b2	10	53.8	653.8
11	48	15-7	b2	9	43.8	583.8
11	51	20-7	b1	9	55.2	595.2

continued...

APPENDIX - IV.a.

Protocol starting time		Date	Male	'True protocol time' (-start & -end state) min sec = seconds		
"EARLY AFTERNOON"				total = 8720.7		
12	0	11-7	b11	10	4.8	604.8
12	4	15-7	b11	12	7.2	727.2
12	5	20-7	b4	11	4.8	664.8
12	7	23-7	b11	12	38.9	758.9
12	15	11-7	b6	5	32.7	332.7
12	27	11-7	b8	10	20.1	620.1
12	29	23-7	b9	10	50.4	650.4
12	43	11-7	b4	9	51.1	591.1
12	45	23-7	b14	7	48.4	468.4
12	50	11-7	b1	8	15.1	495.1
12	59	23-7	b1	2	48.3	168.3
13	3	05-8	b20	9	19.4	559.4
13	16	05-8	b11	12	32.7	752.7
13	43	05-8	b6	6	16.5	376.5
14	55	24-7	b11	15	50.3	950.3
"LATE AFTERNOON"				total = 15608.5		
15	6	16-7	b2	10	44.2	644.2
15	18	24-7	b6	12	15.7	735.7
15	20	16-7	b11	10	39.2	639.2
15	35	24-7	b4	0	2.1	2.1
15	37	16-7	b6	11	20.9	680.9
15	47	24-7	b1	8	43.5	523.5
15	53	16-7	b4	11	56.6	716.6
15	55	26-7	b11	9	31.3	571.3
16	5	26-7	b20	9	2.1	542.1
16	8	16-7	b3	11	27.3	687.3
16	18	23-7'	b11	9	49.3	589.3
16	21	16-7	b1	4	1.2	241.2
16	30	26-7	b6	7	49.7	469.7
16	45	17-7'	b2	10	4.3	604.3
16	52	18-7'	b11	11	58.7	718.7
16	56	09-8	b15	11	14.8	674.8
16	58	17-7'	b11	9	11.6	551.6
16	58	19-7'	b11	10	27.3	627.3
17	3	23-7'	b6	10	20.8	620.8
17	13	18-7'	b6	12	17.5	737.5
17	13	19-7'	b6	11	49.4	709.4
17	24	17-7'	b4	10	5.3	605.3
17	30	19-7'	b1	10	18.8	618.8
17	31	18-7'	b1	12	1.2	721.2
17	41	09-8	b6	4	2.9	242.9
17	42	19-7	b4	13	40.1	820.1
17	56	19-7'	b14	5	18.8	318.8

continued...

APPENDIX - IV.a.

Protocol starting time		Date	Male	'True protocol time' (-start & -end state) min sec = seconds		
"EVENING"				total = 6401		
		18 5	05-7'	b2	7 53.1	473.1
		18 10	19-7'	b9	9 35	575
		18 11	08-8	b20	7 36.8	456.8
		18 17	05-7'	b11	7 21	441
		18 24	08-8	b11	0 19.9	19.9
		18 32	05-7'	b6	9 40.8	580.8
		18 37	08-8	b15	7 35.7	455.7
		18 43	05-7'	b8	9 17.4	557.4
		19 6	06-8	b20	0 28.3	28.3
		19 28	06-8	b11	16 6.9	966.9
		19 51	06-8	b6	9 18.9	558.9
		20 20	05-8'	b20	0 30.9	30.9
		20 32	05-8'	b11	10 36.2	636.2
		20 55	05-8'	b6	10 20.1	620.1

' = second dive

Appendix IV.b. Total duration of each state, expressed as a percentage over the total protocol time, for each of the daytime categories.

DURATION (IN PERCENTAGES)

State	MORNING	Late	AFTERNOON	Late	EVENING
	Early		Early		
	06:00 - 09:00		12:00 - 15:00		
		09:00 - 12:00		15:00 - 18:00	18:00 - 21:00
1	0.75	0.06	0.76	2.19	0.86
2	61.62	64.65	71.82	65.68	41.19
3	15.32	10.72	8.23	15.48	17.6
4	0.34	0.27	0.04	0.04	0.66
5	0.27	0.65	0.15	0.02	0
6	20.44	23.38	18.2	16.44	39.5
7	0.07	0.12	0.17	0.15	0.19
8	0	0.09	0.6	0	0
9	0.12	0.06	0.03	0	0
10	1.07	0	0	0	0
Total (%)	100	100	100	100	100
(secs)	12822.	8856.2	8720.7	15608.	6401

The data is expressed in percentages.

Appendix IV.c. Frequencies of each event, summarised and expressed per hour of total protocol time, for each of the five daytime categories.

FREQUENCIES OF OCCURRENCE

Event	MORNING Early	Late	AFTERNOON Early	Late	EVENING
	06:00 - 09:00	09:00 - 12:00	12:00 - 15:00	15:00 - 18:00	18:00 - 21:00
1	6.5	2	4.1	5.3	1.7
2	72.1	80.9	69.7	70.3	50.6
3	5.9	0.4	0	0	0
4	9.5	10.2	16.5	26.8	23.6
5	0.6	0.4	0	3.7	0.6
6	1.4	0.8	0.4	0	0
Total (hours)	3.5618	2.4600	2.4224	4.3356	1.7780

The data is expressed in frequencies per hour.