Reproductive Strategies of the Insidious Fish Ectoparasite, *Neobenedenia* sp. (Capsalidae: Monogenea)

Truong Dinh Hoai1,2, Kate S. Hutson1*

1 Marine Parasitology Laboratory, Centre for Sustainable Tropical Fisheries and Aquaculture and the School of Marine and Tropical Biology, James Cook University, Queensland, Australia, 2 Aquatic Environment and Fish Pathology Department, Faculty of Animal Science and Aquaculture, Vietnam National University of Agriculture, Hanoi, Vietnam

**Abstract**

Fish monogeneans are lethal parasites in aquaculture. We provide the first experimental evidence that a notorious fish monogenean, *Neobenedenia* sp., can produce viable eggs in isolation for three consecutive generations. We infected individual, isolated, farmed barramundi, *Lates calcarifer* (Bloch) with a single oncomiracidium (larva) of the hermaphroditic monogenean *Neobenedenia* sp. Isolated parasites reached sexual maturity at day 10 post-hatch (24°C, 35%) and laid ~3,300 embryonated eggs over 17 days. Egg production rapidly increased following sexually maturity on day 10 (58 ± 15 eggs) and peaked on day 15 (496 ± 68 eggs) before gradually decreasing. *Neobenedenia* sp. exhibited egg laying and egg hatching rhythms. Parasites laid eggs continuously, but egg production increased in periods of darkness (64.3%), while the majority of oncomiracidia (81%) emerged from eggs in the first three hours of light. Eggs laid by isolated ‘parent’ parasites hatched and individual emerging oncomiracidia were used to infect more individual, isolated fish, with three consecutive, isolated, parasite generations (F1, F2 and F3) raised in the laboratory. Infection success and egg hatching success did not differ between generations. Our data show that one parasite, in the absence of a mate, presents a severe threat to captive fish populations.

**Introduction**

Monogeneans exhibit sophisticated life history strategies in order to ensure their survival in contrasting and unpredictable environments. Evolutionary strategies include multiple reproductive mechanisms, predator avoidance and behavioural responses to host and environmental cues that favour enhanced infection success. In wild populations these strategies ensure some parasites survive to the next generation, whereas in captive populations, where host organisms are confined in high densities, it can lead to parasite epizootics.

Various reproductive mechanisms have been observed in monogeneans including oviparity [1], viviparity [2] and self-fertilisation [3]. Most oviparous monogeneans deposit fewer than 100 eggs/parasite/day [4–7], although some species can produce more than 550 eggs/parasite/day [8]. In viviparous monogeneans, up to three consecutive generations can develop inside the mother parasite. For example, a single individual *Gyrodactylus salaris* bears the first daughter within 24 hours of the birth of the parent [9] and thus has the capacity to produce six million offspring in four weeks [1]. Self-fertilisation is known to occur in monogenean species that infect the bladder of amphibians [3,10] and ensures reproductive potential when a parasite finds itself alone on a host. Monogeneans also exhibit egg laying and egg hatching rhythms, which can reduce the risk of predation and coincide with host behaviours to ensure infection success. Monogeneans maximise their chances of finding a host by releasing eggs into the environment during certain times of the day or night [7], extending the hatching period [11], responding to hatching cues such as shadows [12], chemicals [11,13], mechanical disturbance [14–16] and osmotic changes [17], most of which are generated by the host.

*Neobenedenia* are marine capsalid monogeneans of critical concern to aquaculture because they exhibit several life history traits that aid their survival. *Neobenedenia* spp. have direct life cycles with short generation times [18,19] and low host specificity [20,21] which has resulted in major stock losses in several aquaculture fish species (see [18,22–24]). Furthermore, attached parasite stages are transparent, which can reduce predation by cleaner organisms [21]. Eggs are encapsulated by a proteinaceous shell which confers protection to the developing embryo from digestion [25], most chemicals [26–29] and bacteria [1]. Capsalid monogeneans are hermaphrodites, displaying several types of reproduction including mutual cross-insemination [30], attach-
ment of spermatophores to other individuals [31] and self-
semination (as observed by the copulatory organ lodged in the
parasite’s own uterus; see [30,32]). However, no studies have
experimentally examined whether fish monogeneans can success-
fully reproduce in isolation and produce viable eggs and larvae.

The aim of this research was to experimentally examine the
reproductive strategies of Neobenedenia. Specifically we sought to
determine: 1) whether Neobenedenia can reproduce in isolation; 2) Neobenedenia fecundity over time; 3) whether Neobenedenia
exhibit egg laying rhythms and/or hatching rhythms. We used a
barramundi, or Asian seabass, *Lates calcarifer* – Neobenedenia sp.
model system for our experiments. Barramundi (*Perciformes: Latidae*) are distributed in estuaries and coastal seas from south-
western India to north-eastern Australia, with approximate
lattitudes of ±25° [33]. This species is among the most important
food fishes in tropical Australasia and is farmed throughout eastern
and western Asia (China, India, Israel, Indonesia, Malaysia,
Philippines, Singapore, Tahiti, Taiwan, Thailand) and Australia
[34]. High intensities of *Neobenedenia* on farmed fish damage host
epidermis through attachment and feeding [19,23,35] and
increase secondary infections [36,37].

**Materials and Methods**

**Ethics Statement**

This work was conducted using a barramundi, *Lates calcarifer* –
Neobenedenia sp. model system with all procedures approved by
the James Cook University Animal Ethics Committee (A1579).
Neobenedenia sp. used in experiments were collected from private
land in north Queensland, Australia. Future permissions should be
directed to Coral Coast Barramundi Pty Ltd.

**Source of Animals**

Hatchery reared freshwater *L. calcarifer*; mean size
125±23 mm) were purchased from Good Fortune Bay Hatchery,
Queensland, Australia for use in experiments 1–4. Fish were not
previously exposed to *Neobenedenia*. Fish were transported to the
laboratory in 50 L tanks with air supplied through battery
powered aerators and held in fresh water in 100 L aquaria until
required. Fish were acclimated to sea water 48 h prior to
experiments by increasing salinity to 0, 10, 20, 30 and 35‰ over
6 h intervals. Sea water used in experiments was UV treated,
10 μm filtered, 35‰, unless stated otherwise.

*Neobenedenia* sp. used in experiments were collected from a
land-based marine *L. calcarifer* farm (Coral Coast Barramundi
Pty Ltd) in north Queensland, Australia. An infection was
maintained on *L. calcarifer* (size range 110-220 mm) held in
100 L marine aquaria to provide a continuous source of parasites.
Neobenedenia sp. (hereafter as Neobenedenia) investigated in this
study is presently unidentified given the absence of diagnostic
criteria to differentiate between geographical/host isolates and
species [20,21,38] [20,21,33]. Representative parasites were
acceded in the Australian Helminth Collection (AHC) at the
South Australian Museum (SAMA AHC 35461; see [39]).

**Experiment 1: Reproduction of Isolated Neobenedenia**

To determine whether hermaphroditic *Neobenedenia* can
reproduce in isolation, individual, isolated fish were infected with
a single oncomiracidium (larva). Oncomiracidia were sourced from
embryonated *Neobenedenia* eggs collected from the labora-
tory infection. Eggs were incubated in glass cavity blocks in sea
water at 25°C in culture chambers on a 12:12 h LD cycle (Sanyo:
ML-351 Versatile Environmental Incubation Chamber). A third
of the solution (2 mL) was exchanged every 24 h and eggs were
monitored daily under a stereomicroscope using both transmitted
and incident light. When eye spots were observed in the eggs,
monitoring was increased to every 2 h in order to obtain newly
hatched oncomiracidia. Individual oncomiracidia <4 h old were
gently aspirated using a fine-tip glass pipette under a stereo-
microscope and introduced to a 10 L aquarium containing an
individual *L. calcarifer* in 6 L of sea water. Each oncomiracidium
was released at the bottom of the aquarium to avoid the effects of
surface tension and currents which can trap and kill oncomiracidia
[1]. When the oncomiracidia were introduced, air supply to the
fish was stopped for 1 h in order to reduce water currents
and thereby increase infection success [19]. Thirty replicates were
made at room temperature (24.3±0.2°C). Salinity in each
aquarium was checked using a refractometer every 24 h and
adjusted by adding distilled water to maintain 35±1‰.

To determine the onset of egg production, a piece of 5 cm² fine
gauge (0.5×0.5 mm) netting was immersed in each aquarium and
checked daily under a stereomicroscope. Eggs have filamentous
strings which easily entangle on netting [40]. The day that eggs
were observed on the netting was recorded as time to sexual
maturity. Eggs always entangle on netting on the day of sexual
maturity (AK Brazenor, unpublished data). Netting was renewed
daily and any suspended eggs remaining in the sea water were
collected daily by filtering the solution through a 60 μm mesh.

In order to confirm isolated fish were infected by a single,
individual parasite, fish were immersed in fresh water at the end of
the experiment, which kills *Neobenedenia* [see 35]. Fish were
bathed in 1 L of fresh water containing a mild sedative (Aquari-S
1:1000) for 5 min. The fresh water solution and the body surface
of the fish were examined twice under a stereomicroscope to
collect detached and attached parasites, respectively.

We sought to determine the reproductive viability of consecu-
tive generations of reproducitively isolated parasites for a single
parasite lineage. Eggs laid by a randomly selected isolated ‘parent’
parasite were incubated in culture chambers (25°C, 35‰). A single
oncomiracidium from these eggs was used to infect each of 15
replicate, isolated *L. calcarifer*. This process was repeated to infect
10 and 30 fish using oncomiracidia from generation F1 and F2,
respectively (Table 1). Infection success was recorded as the
number of oncomiracidia that attained sexual maturity from the
number of fish challenged.

**Experiment 2: Egg Hatching Success**

In order to assess the viability of eggs laid by isolated parasites,
egg hatching success was determined for a single parasite lineage
for three consecutive, reproducitively isolated, *Neobenedenia*
generations. A total of 30 fish (ten for each generation) were
infected as per the methods in Experiment 1. Infection success was
recorded as the number of oncomiracidia that attained sexual
maturity from the number of fish challenged. *Neobenedenia* eggs
were collected by filtering the aquarium sea water through a
60 μm filter three days following sexual maturity. Following egg
collection, individual fish were bathed in fresh water to confirm
infection by an individual parasite (see Experiment 1). Clusters of
eggs (containing 8–46 individual eggs) were incubated in sea water
in cavity blocks in laboratory conditions (natural light,
24.3±0.1°C) with six replicate egg clusters made for each infected
fish. Cavity blocks were filled with sea water to the brim
and covered with a glass cover. Blocks were monitored for egg
hatching every 24 h, when one third of the solution was changed
with minimal disturbance to the eggs. When hatching was
observed, oncomiracidia were removed with a pipette. Hatching
experiments were continued until 48 h passed without hatching in
any treatment. Hatching success of *Neobenedenia* eggs was
measured as the number of oncomiracidia removed divided by the total number of eggs.

**Experiment 3: Fecundity of Isolated and Cross-fertile Neobenedenia**

To determine the fecundity of isolated parasites, egg production was monitored every 24 h. Twenty-five fish were infected with an individual oncomiracidium sourced from the laboratory infection (see Experiment 1). All aquaria (10 L with 6 L sea water) were maintained in laboratory conditions (24.3±0.1°C, 35±1‰). Ten fish (40%) were successfully infected. Daily egg production was determined by filtering the aquarium sea water through a 60 μm filter at 1800 each day. Immediately prior to filtering, fish were gently removed by hand and placed into a new aquarium containing fresh sea water. Eggs were counted under a stereomicroscope using a hand held counter to determine egg production/parasite/day. The filtered sea water was also examined carefully for parasites. The experiment was terminated when mean egg production was <50 eggs/day for two consecutive days. At the termination of the experiment, individual fish were bathed in fresh water to confirm infection by an individual parasite (see Experiment 1).

To determine the fecundity of parasites given an opportunity to cross-inseminate, egg production was monitored every 12 h for fish infected with multiple oncomiracidia. Groups of ten oncomiracidia were used to challenge three fish, maintained in three separate aquaria in laboratory conditions. Egg production was monitored every 12 h (at 0600 and 1800). The experiment was terminated when mean egg production was <50 eggs/day for two consecutive days. On the day the experiment was terminated, fish were bathed in fresh water and parasites were counted. Egg production in each replicate was divided by the total number of parasites recovered to determine mean number of eggs laid per parasite.

**Experiment 4: Egg Laying Rhythm**

To determine whether Neobenedenia exhibit an egg laying rhythm, egg production was monitored every 3 h for three days. Four fish infected with an individual, isolated Neobenedenia, were monitored between day 12 and 15 post-infection. Ten litre aquaria containing individual infected fish were placed in culture chambers on day 10 post-infection on a 12:12 h LD cycle at 25°C. Aquaria were aerated with battery operated aerators and salinity was maintained at 35±1‰. Egg production was determined every 3 h for three days (72 h) by filtering the aquarium sea water through a 60 μm filter, commencing at the first period following the onset of darkness (1800–2100) on day 12. Immediately prior to filtering, fish were gently removed by hand and placed into a new aquarium with fresh sea water. Eggs were counted under a stereomicroscope using a hand held counter to determine egg production/parasite/3 h. At the termination of the experiment, individual fish were bathed in fresh water to confirm infection by an individual Neobenedenia (see Experiment 1).

**Table 1. Infection success and egg hatching success of three consecutive isolated Neobenedenia sp. generations infecting Lates calcarifer.**

| Source of oncomiracidia | Infection success (%) | Parasite generation | Egg hatching success (%) |
|-------------------------|-----------------------|---------------------|-------------------------|
| Embryonated eggs (laboratory infection) | 35 (n = 40) | Parent | 78 (n = 42) |
| From eggs laid by isolated parent parasites | 44 (n = 25) | F1 | 75 (n = 30) |
| From eggs laid by isolated F1 parasites | 35 (n = 20) | F2 | 86 (n = 12) |
| From eggs laid by isolated F2 parasites | 56 (n = 30) | F3 | Not examined |

The number of replicates (n) is indicated in parentheses.

*Data combined from Lates calcarifer infected in Experiment 1 and 2.

doi:10.1371/journal.pone.0108801.t001

Statistical Analysis

A chi-square distribution was used to examine proportional infection success between consecutive isolated generations. Egg hatching data were analysed by permutational analysis of variance in the PERMANOVA function of PRIMER 6.0. PERMANOVA compares the observed value of a test statistic (F-ratio) against a recalculated test statistic generated from random permutation of
Egg production rapidly increased from 66 eggs/parasite over the course of the experiment (15 days), with 68.3% of eggs produced during periods of darkness (Fig. 1). Following fresh water bathing, all experimental fish were confirmed to be infected with a single, individual Neobenedenia.

Fecundity
Individual, isolated Neobenedenia were fecund and produced 3,229±37 eggs/parasite over the course of the experiment (17 days of egg-laying; or 8±2 eggs/parasite/hour (e/p/h) or 190±11 eggs/parasite/day (e/p/d)). All parasites reached sexual maturity on day 10 post-infection (at 24.2±0.1°C). Egg production rapidly increased from the day of sexual maturity (58±15 on day 10) and peaked on day 15 (496±68) post-infection (Fig. 1). Following day 16, egg production gradually decreased, to almost negligible egg production on day 23 and 26. A total of eight fish mortalities occurred over the course of the experiment with 9, 8, 6, 3 and 2 fish remaining on days 12, 13, 20, 24 and 26, respectively. No dead, detached parasites were observed in filtered sea water for the duration of the experiment. Following fresh water bathing, all experimental fish were confirmed to be infected with a single, individual Neobenedenia.

There was no significant difference between fecundity of isolated parasites and fish infected with multiple parasites (p = 0.44). Three fish challenged with multiple oncomiracidia (n = 0) were successfully infected with three, three and four parasites, respectively. Neobenedenia with the opportunity to cross-inseminate produced 8±1 e/p/h or 191±22 e/p/d or 2,865±77 eggs/parasite over the course of the experiment (15 days), with 64.3% of eggs produced during periods of darkness (Fig. 2). Parasites reached sexual maturity on day 10 post-infection (Fig. 2). Egg production rapidly increased from 66±5 on day 10 to 49±43 on day 15. On day 16, egg production began to gradually decrease, prior to almost negligible egg production on day 23 and 24 (Fig. 2). No fish mortalities occurred during the experiment, and there were no dead, detached parasites observed in filtered sea water for the duration of the experiment. Parameter estimates for isolated and cross-fertile individuals modelled separately are shown in Table 2. Although there was no significant difference in average fecundity between groups, the model better described the cross-fertile group ($R^2 = 0.93$) than the isolated group ($R^2 = 0.585$). Comparing the residuals of each model with a Levene’s test indicated that the residual variance was significantly higher in the isolated group than the cross-fertile group (p = 0.0048).

Egg Laying Rhythm
Neobenedenia laid eggs continuously, but exhibited a distinct egg-laying rhythm with more eggs laid during periods of darkness. Egg production began gradually increasing in the 3 h period prior to darkness and during periods of darkness, peaking between midnight and 0300 (Fig 3). Production decreased in the 3 h period prior to illumination and the lowest egg production was between midday and 1500 (Fig. 3). Each parasite laid an average of 22±4 eggs/h in periods of darkness and 12±2 eggs/h in periods of light between day 12 and 15 post-infection.
Egg Hatching Rhythm

*Neobenedenia* exhibited a distinct hatching rhythm (p<0.001) with the majority of oncomiracidia (81%) hatching in the first 3 h of light (Fig. 4). Eggs began hatching on day 5, with the majority of hatching occurring on days 7 and 8. A total of 216 embryonated eggs were incubated and all eggs hatched during periods of natural light (0600–1500) with no eggs hatching between 1500 and 0600 on any day/night (Fig. 4).

Discussion

This study provides unambiguous experimental evidence that the fish monogenean, *Neobenedenia* sp., can successfully reproduce in isolation. One isolated *Neobenedenia* has the capacity to produce more than three thousand eggs that hatch into infective larvae within two weeks (Fig. 1), revealing that low *Neobenedenia* burdens in host populations do not necessarily restrict reproductive potential. Furthermore, the progeny of isolated parasites are viable for at least two more consecutive isolated generations (Table 1). While inbreeding tends to decrease hatching and infection success and the genetic diversity of parasite populations [43], we found no significant difference in egg hatching or infection success in consecutive isolated generations (Table 1). Moreover, the nested factor of fish was significant, indicating that the relationship between parasites and their individual hosts is an important aspect in determining parasite reproductive success.

Self-fertilisation is a strategy commonly seen in parasitic platyhelminths where low parasite burdens occur in host populations or where there may be a high frequency of single parasite infection [6,44,45]. At least four species of monogeneans in the bladders of amphibians are capable of producing viable eggs in isolation [3,6,10]. It is possible that *Neobenedenia* examined in this study reproduced by natural parthenogenesis (a form of asexual production where growth of embryos can occur without fertilisation); however, this is unlikely considering that *Neobenedenia* have reproductive organs of both sexes. Kearn and Whittington [30] observed two capsalid monogenean species, *Benedeniella macrocolpa* and *B. posterocolpa*, self-inseminating in preserved specimens mounted on slides, but insemination via the vaginal route is not an option in *Neobenedenia* spp. as there is no vagina. Insemination in *Neobenedenia* spp. is most likely achieved by sperm being introduced via the common genital pore or the uterus. Indeed, Whittington and Horton [20] observed the penis of one *Neobenedenia melleni* lodged in its own uterus. Self-insemination has been observed in live specimens of *Neobenedenia girellae* (see [46]) and *Heterobothrium okamotoi* (Monogenea: Diclidophoridae) (see [32]). Furthermore, Ogawa et al. [46] suggested that self-insemination in *N. girellae* may involve passage of sperm through the tegument from externally attached spermatophores. While the specific mechanism of self-insemination was not determined, our study provides the first experimental evidence that capsalid monogeneans of fish can produce viable eggs in isolation.

Parasites that exhibit high fecundity increase the likelihood of offspring successfully locating and infecting a new host. Isolated *Neobenedenia* were fecund, with egg production rapidly increasing...
following sexual maturity, before peaking and slowly decreasing over time (Fig. 1). This trend is typical of many invertebrate species [47,48]. *Neobenedenia* egg production was negligible by the time adult parasites were ~23 days old, indicating that fecundity was captured over the reproductive life span of the parasite (Fig. 1 and 2). Egg production varied with parasite age and time of day, indicating that egg production measured on an hourly or daily rate may not accurately represent parasite fecundity [49]; Fig 1; Fig 3). Egg production can also be influenced by other environmental and host variables, including temperature [50–52].

Parasite fecundity can vary between self-fertile and cross-fertile individuals. Wedekind et al. [53] reported that self-fertile cestodes, *Schistcephalus solidus*, infecting stickleback, *Gasterosteus aculeatus*, exhibited higher fecundity compared to parasites placed in pairs. In contrast, Tinsley and Owen [3] found that multiple infections of the monogenean *Protopolystoma xenopodis* infecting the toad, *Bufo regularis*, sometimes resulted in greater output per individual than isolated parasites. In our study, there was no significant difference in fecundity for isolated and cross-fertile *Neobenedenia*. It is plausible that *Neobenedenia* may not have cross-fertilised, despite being infected with multiple individuals and their ability to ‘crawl’ along the external surfaces of fishes in order to locate another parasite [54]. Thus, molecular methods are warranted to quantify the frequency of cross-fertilisation in *Neobenedenia*

Parasite egg laying rhythms could be a predator avoidance behaviour and could also align with temporal host behaviours [25]. *Neobenedenia* laid eggs continuously, but significantly more eggs (64.3%) were laid at night (Fig. 3 & 4). Egg-laying rhythms are common in many invertebrates, with most releasing their gametes during periods of darkness (Fig. 3; [55,56,57]). The egg laying rhythm of *Diplorhynchus hippoglossi* (Monogenea: Diplozoidae), a gill parasite of southern barbel, *Barbus meridionalis*, is also nocturnal [7]. Similarly, Mooney et al. [51] found that *Heteraxine heterocerca* (Monogenea: Heteraxinae) a gill parasite of Japanese yellowtail, *Seriola quinqueradiata*, laid eggs continuously, but more eggs (72.9%) were laid during periods of darkness, with the majority of eggs released during the first 3 h periods immediately after dark. Alternatively, some monogenean species store their eggs in *utero* to be released at a specific time of day [8,51].

Hatching rhythms can also increase the chances of larvae contacting a specific host [58,54]. This is crucial for infection success because free swimming oncomiracidia are typically short lived (24 to 48 h) [1,26,59]. *Neobenedenia* exhibited a distinct hatching rhythm, with 81% of the larvae emerging during the first 3 h of natural light (Fig. 4). Monogeneans can maximise their chances of finding a host by extending the hatching period [11], while some species respond to hatching cues such as shadows [12], chemicals [11,13], mechanical disturbance [14–16] and osmotic changes [17], most of which are generated by the host. Hatching rhythms have been documented in other marine monogeneans in the first few hours of light (*Entobdella solea* [60]; *Dichlidophora* spp. [15]) and also in the first few hours following dusk (*Entobdella hippoglossi* [61]; *Diplorhynchus hippoglossi* [7]). Other species exhibit more complicated rhythms [41,58,62,63]. Hatching rhythms of monogeneans are often related to times when the behaviour of the host makes it more vulnerable to infection [7,15,59–62]. Eggs also hatch in response to other environmental cues such as light periodicity and intensity [60,64], host skin secretions [65], mechanical disturbances [66] and seasonal differences [15]. In addition, rhythmic hatching may minimise predation on monogeneans by other organisms, especially filter feeders [58,63].

In conclusion, *Neobenedenia* exhibits a variety of strategies to aid survival of subsequent generations. Parasites can reproduce in isolation to produce viable, infective oncomiracidia for three consecutive generations. High fecundity, egg laying and egg hatching rhythms ensure the success and persistence of this harmful parasite in wild and farmed fishes.

**Acknowledgments**

We thank Alexander Brazenor, Thane Miltitz and Alejandro Trujillo-Gonzalez (JCU) for technical assistance and Good Fortune Bay Pty Ltd and Coral Coast Barramundi Pty Ltd for provision of specimens. Emeritus Professor Rhondda Jones and Dr Richard Saunders (JCU) provided statistical advice and Associate Professor Ian Whittington (South Australian Museum) provided discussion on monogenean biology.

**Author Contributions**

Conceived and designed the experiments: KSH. Performed the experiments: TDH. Analyzed the data: TDH KSH. Contributed reagents/materials/analysis tools: KSH. Wrote the paper: KSH TDH.

**References**

1. Whittington ID, Chisholm LA (2008) Diseases caused by *Monogenea*. In: Eiras JC, Seguer H, Wahlii T & Kapoor BG Editors. Fish Diseases. Science Publishers, Inc, New Hampshire, USA. pp. 683-816.
2. Harris P, Tinsley R (1987) The biology of *Gyrodactylus galliwei* (Gyrodactyloidea), an unusual viviparous monogenean from the African clawed toad, *Xenopus laevis*. Journal of Zoology 212: 325–346.
3. Tinsley R, Owen RW (1975) Studies on the biology of *Protopolystoma xenopodis* (Monogeneida): the oncomiracidium and life-cycle. Parasitology 71: 445–463.
4. Tinsley R (1993) Ovoviviparity in platyhelmint life-cycles. Parasitology 86: 161–196.
5. Kearn G (1985) Observations on egg production in the monogenean *Entobdella solea*. International Journal for Parasitology 15: 187–194.
6. Jackson HC, Tinsley R (1988) The capacity for viable egg production by the monogenean *Protopolystoma xenopodis* in single and multiple infections. International Journal for Parasitology 18: 585–589.
7. Macdonald S, Jones A (1978) Egg-laying and hatching rhythms in the monogenean *Diplorhynchus hippoglossi* from the southern barbel *Barbus meridionalis*. Journal of Helminthology 52: 23-28.
8. Mooney AJ, Ernst I, Whittington ID (2006) An egg-laying rhythm in *Zenuatia servoidae* (Monogenea: Heteraxinae), a gill parasite of yellowtail kingfish (*Seriola lalandii*). Aquaculture 253: 10–16.
9. Scott ME (1982) Reproductive potential of *Gyrodactylus belluardus* (*Monogenea*) on guppies (*Poecilia reticulata*). Parasitology 85: 217–236.
10. Combes C (1972) Influence of the behaviour of amphibians on helminth life cycles. Zool J Linn Soc 51: 151–170.
11. Macdonald S (1974) Host skin mucus as a hatching stimulant in *Acanthobdella lobanchi*, a monogenean from the skin of *Raja spp*. Parasitology 68: 331–338.
12. Gannicott A, Tinsley R (1997) Egg hatching in the monogenean gill parasite *Dincoyctus sargittatus* from the rainbow trout (*Oncorhynchus mykiss*). Parasitology 114: 569–579.
13. Whittington I (1987) Hatching in two monogenean parasites from the common dogfish (*Scyliorhinus canicula*): the polyphophycocytellid gill parasite, *Heubrochius appendiculatum* and the microbothrid skin parasite, *Leptocotyle minor*. Journal of the Marine Biological Association of the United Kingdom 67: 729–736.
14. Glennon V, Chisholm LA, Whittington ID (2006) Three unrelated species, 3 sites, same host–monogenean parasites of the southern fiddler ray, *Trygonorrhina fasciata*, in South Australia: egg hatching strategies and larval behaviour. Parasitology 133: 55–66.
15. Macdonald S (1975) Hatching rhythms in three species of *Dichlidophora* (Monogenea) with observations on host behaviour. Parasitology 71: 211–228.
16. Whittington ID, Cribb BW, Hamwood TE, Halliday JA (2000) Host-specificity of monogeneans (platyhelmint) parasites: a role for anterior adhesive areas? International Journal for Parasitology 30: 305–320.
17. Tinsley R (1978) Oviposition, hatching and the oncomiracidium of *Eupolyxotoma antoria* (*Monogeneidae*). Parasitology 77: 121–132.
18. Hirazawa N, Mitsuishi T, Hirata T, Shiras K (2004) Susceptibility of spotted halibut *Varius variatus* (*Pleuronectidae*) to infection by the monogenean *Neobenedenia girellae* (Capitidae) and oral therapy trials using praziquantel. Aquaculture 238: 83–95.

PLOS ONE | www.plosone.org 6 September 2014 | Volume 9 | Issue 9 | e108801
19. Hirazawa N, Takano R, Hagiwara H, Noguchi M, Narita M (2010) The influence of different water temperatures on Neobenedenia girellicae (Monogenea) infection, parasitic growth, egg production and emerging second generation on amberjack Seriola dumerilli (Carangidae) and the histopathological effect of this parasite on fish skin. Aquaculture 299: 2-7.

20. Whittington I, Horton M (1996) A revision of Neobenedenia Yamaguti, 1963 (Monogenea: Capsalidae) including a redescription of N. melleni (MacCallum, 1927) Yamaguti, 1963. Journal of Natural History 30: 1113–1156.

21. Whittington ID, Myraveewa S, Blandford S, de Silvester VA, Harvey AW (2002) Benedenia seriolae and Neobenedenia species. In: Woo, PTK and Buchmann, K editors. Fish Parasites: Pathobiology and Protection. CAB International, Wallingford, Oxfordshire, UK. pp 225-244.

22. Devey MR, Chisholm LA, Whittington ID (2001) First published record of the pathogenic monogenean parasite Neobenedenia melleni (Capsalidae) from Australia. Diseases of Aquatic Organisms 46: 79-82.

23. Ogawa K, Miyaizato J, Wang HC, Lo CF, Kou GH (2006) Neobenedenia girellicae (Monogenea) infection of cultured cobia Rachycentron canadum in Taiwan. Fish Pathology 41: 51–56.

24. Ogawa K, Yokoyama H (1998) Parasitic diseases of cultured marine fish in Japan. Fish Pathology [Japan] 33: 303–309.

25. Kearn GC (1986) Eggs of monogeneans. Advances in Parasitology 25: 275–273.

26. Milita TA, Southgate PC, Carton AG, Huston KS (2014) Efficacy of garlic (Allium sativum) extract applied as a therapeutic immersion treatment for Neobenedenia sp. management in aquaculture. Journal of Fish Diseases 37: 451–461.

27. Thoney D (1990) The effects of trichlorfon, praziquantel and copper sulphate on the cownose ray, Rhinoptera bonasus (Mitchill). Journal of Fish Diseases 13: 385–389.

28. Wharton D (1983) The production and functional morphology of helminth egg-shells. Parasitology 96: 85–97.

29. Yoshinaga T, Segawa I, Kamiishi T, Sorimachi M (2000) Effects of temperature, salinity and chlorine treatment on egg hatching of the monogenean Neobenedenia thunzi infecting Japanese flounder. Fish Pathology 35: 85–86.

30. Kearn GC, Whittington I (1992) Diversity of reproductive behaviour in platyhelminth parasites: insinuation in some benedenid (capsalid) monogeneans. Parasitology 104: 489–496.

31. Kearn G (1970) The production, transfer and assimilation of spermatophores by Entobdella solanum, a monogenean parasite of the common sole. Parasitology 60: 301–311.

32. Ogawa K (2002) Impacts of dichlofluanid monogenean infections on fisheries in Japan. International Journal for Parasitology 32: 373–380.

33. Prithivajeera R, Gill AC (2013) Taxonomy and Distribution of Indo-Pacific Lates Biology and Culture of Asian Seabass I-I. CRC Press, Taylor and Francis Group, USA, pp 1–15.

34. Huston KS (2013) Infectious diseases of Asian seabass and health management. In: Jerry DR Editor. Biology and Culture of Asian Seabass. CRC Press, Taylor and Francis Group, USA. pp 102–136.

35. Kaneko JJ, Yamada R, Brock J, Nakamura R (1988) Infection of ulopa, Oteroichromis mossambicus (Trewavas), by a marine monogenean, Neobenedenia mellicanii (MacCallum, 1927) Yamaguti, 1963 in Kanoehe Bay, Hawaii, USA, and its treatment. Journal of Fish Diseases 11: 295–300.

36. Thoney D, Hargis W Jr (1991) Monogenea (Platyhelminthes) as hazards for fish aquaculture. Journal of Fish Diseases 14: 59–70.

37. Whittington ID, Kearn GC (1989) Rapid hatching induced by light intensity from the frog skin parasite Entobdella hippoglossi infecting the Common Sole. Parasitology 124: 423–434.

38. Kearn G, Ogawa K, Maeno Y (1992) The oncomiracidium of Heterotexa heterocerca, a monogenean gill parasite of the yellowtail Seriola quinquemaculata. Publications of the Seto Marine Biological Laboratory 35: 347–350.

39. Mooney AJ, Ernst I, Whittington I (2008) Egg-laying patterns and in vivo egg production in the monogenean parasites Heterotexa heterocerca and Benedenia seriolae from Japanese yellowtail Seriola quinquemaculata. Parasitology 135: 1293–1302.

40. Tabbs I, Poestenaar C, Sewell M, Diggles B (2005) Effects of temperature on fecundity in vitro, egg hatching and reproductive development of Benedenia seriolae and Xenotroctra seriolae (Monogenea) parasite on yellowtail kingfish Seriola lalandi. International Journal for Parasitology 35: 313–327.

41. Wedekind C, Strahm D, Scharer L (1998) Evidence for strategic egg production in a hermaphroditic cestode, Parsitology 114: 373–382.

42. Whittington ID, Ernst I (2002) Migration, site-specificity and development of Benedenia latijunta (Monogenea: Capsalidae) on the surface of its host, Lutjanus carponotatus (Pisces: Lutjanidae). Parasitology 124: 423–434.

43. Kearn G, Ogawa K, Maeno Y (1992) The oncomiracidium of Heterotexa heterocerca, a monogenean gill parasite of the yellowtail Seriola quinquemaculata. Publications of the Seto Marine Biological Laboratory 35: 347–350.

44. Taylor MH, Leach GJ, DMichele L, Levitan WM, Jacob WF (1979) Lunar spawning cycle in the mummichog, Fundulus heteroclitus (Pisces: Cyprinodontidae). Copeia 1979: 291-307.

45. Kearn G (1973) An endogenous circadian hatching rhythm in the monogenean parasite Entobdella solanum from the gills of the gilthead seabream Sparus aurata. Journal of the Marine Biological Association of the United Kingdom 63: 753–756.

46. Taylor MH, Leach GJ, DMichele L, Levitan WM, Jacob WF (1979) Lunar spawning cycle in the mummichog, Fundulus heteroclitus (Pisces: Cyprinodontidae). Copeia 1979: 291-307.

47. Kearn G (1973) An endogenous circadian hatching rhythm in the monogenean parasite Entobdella solanum, and its relationship to the activity rhythm of the host (Solea solea). Parasitology 66: 101–122.

48. Kearn G (1974) Nocturnal hatching in the monogenean skin parasite Entobdella hippoglossi from the halibut, Hippoglossus hippoglossus. Parasitology 68: 161–172.

49. MacDonald S, Combes C (1978) The hatching rhythm of Polystoma integerrimum, a monogenean from the frog Rana temporaria. Chronobiologia 5: 377.

50. Whittington I, Kearn G (1986) Rhythmic hatching and oncomiracidial behaviour in the hexactinellid monogenean Raphidascobole emarginata from the gills of Raja spp. Journal of the Marine Biological Association of the United Kingdom 66: 93–111.

51. Whittington ID, Kearn GC (1989) Rapid hatching induced by light intensity reduction in the polyopisthocotylean monogenean Plectanomyce gurnardi from the gills of gurnards (Triglidae), with observations on the anatomy and behaviour of the oncomiracidium. Journal of the Marine Biological Association of the United Kingdom 69: 609–624.

52. Kearn GC (1974) The effects of fish skin mucus on hatching in the monogenean parasite Entobdella solanum from the skin of the common sole, Solea solea. Parasitology 68: 173–188.

53. Whittington ID, Kearn GC (1988) Rapid hatching of mechanically-disturbed eggs of the monogenean gill parasite Dichlidophloeus lineatus, with observations on sedimentation of egg bundles. International Journal for Parasitology 18: 847-852.