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Research Note

Does the anaesthetic influence behavioural transmission of the monogenean *Gyrodactylus gasterostei* Gläser, 1974 off the host?

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Summary

The aim of this study was to investigate the use of the anaesthetic 2-phenoxyethanol on the transmission factors of gyrodactylid and to ascertain how this may affect in the colonisation of new hosts using the *Gyrodactylus gasterostei* Gläser, 1974 – *Gasterosteus aculeatus* L. model which is a simple and successful system to examine aspects of transmission of parasites from live and dead fish. Laboratory experiments include determining the maturity (presence of male copulate organ) and reproductive (presence of daughter) status of transmitting worms, in order to consider the factors that influence parasite option to migrate to a new individual of the same host species. This study demonstrates that parasites with a Male Copulate Organ (MCO) present are more likely to abandon the host and attempt a host transfer. The use of the anaesthetic 2-phenoxyethanol does not affect transmission of gyrodactylids which leave the host to colonise a new host. Finally, the use of other anaesthetic although its relative importance with respect to transmission remains uncertain.
Keywords: *Gyrodactylus*; transmission; anaesthetic

Introduction

Gyrodactylids as monogenean flukes have direct life-cycles, are viviparous and are capable of rapid increase in numbers. Gyrodactylids lack a free swimming larval stage or oncomiracidium which is present in egg-laying monogeneans, but instead have developed other highly successful reproductive strategies i.e give birth to full sized living individuals (Bakke *et al*., 2007). Harris (1993) described this mode of reproduction as being fairly unique, allowing rapid population growth on their host and conferring an ability to transfer to a new host at all times during their life-cycle. The skin, gills and oral cavity are the principal surfaces of fish that are in contact with water, offering particularly favourable conditions for the establishment and survival of parasitic animals *i.e* epithelial cells, as food, is much more accessible for gyrodactylids (Bakke *et al*., 2007).
The *G. gasterostei* / *Gasterosteus* model is a simple and successful system to examine aspects of transmission of parasites from live and dead fish, since Harris (1985) and recently, Grano-Maldonado (2011a) employed this model on the biological and behavioural basis of host selection; the accidental gyrodactylid transfer during multiple fish transportation (Grano-Maldonado, 2011b); description of the ultrastructure of sensory organs i.e mechano, chemical and photoreceptors (Grano-Maldonado, 2014a) and oral transmission as a successful transmission strategy (Grano-Maldonado, 2014b).

Material and Methods

A series of different experiments were performed to study aspects of transmission in *G. gasterostei*. Experiments were conducted to assess the behaviour of the parasites under different scenarios: i) choice of anaesthetic and its effect on gyrodactylids transmission and ii) pitting (piercing the head and destroying the brain) was employed as control.

Source of hosts and parasites
An experimental model using the monogenan *Gyrodactylus gasterostei* and the host fish *Gasterosteus aculeatus* was used. Fish used were natural infected and collected from a settlement

Table 1. The developmental status of gyrodactylids transferring from the anaesthetic euthanised fish [a] (n = 20); gyrodactylids remaining on the anaesthetic euthanised fish [b] (n = 20); gyrodactylids transferring from hosts that were killed by pithing [c] (n = 20); gyrodactylids that remained on hosts that were killed by pithing [d] (n = 20), Where N=no; D = daughter; MCO= male copulatory organ.

	Maturity status MCO/D	**N MCO/D**	**MCO/ND**	**N MCO/ND**	**TOTAL**
Number of parasites	8 [a]	14 [a]	23 [a]	2 [a]	47 [a]
SD	0.82	0.73	1.26	0.30	
%	17.02	29.78	48.93	4.25	
Number of parasites	42 [b]	108 [b]	36 [b]	12 [b]	**198** [b]
SD	1.65	3.01	2.015	0.68	
%	21.21	54.54	18.18	6.06	
Number of parasites	15 [c]	12 [c]	22 [c]	12 [c]	**61** [c]
SD	1.2	0.8	1.29	0.68	
%	24.59	19.67	36.06	19.67	
Number of parasites	64 [d]	115 [d]	36 [d]	23 [d]	**238** [d]
SD	3.57	5.01	2.04	1.46	
%	26.89	48.31	15.12	9.66	

pond, feeding a commercial fish farm, situated on a branch of the River Allan near Stirling, Stirlingshire, Scotland (56° 06' 37.77" N, 3° 58' 25.25" W). Fish were transferred to an aquarium facility at the Institute of Aquaculture, University of Stirling, where they were held in 25 L black plastic tanks containing 15 ± 1 °C, aerated "home" stream water. Fish were fed *ad libitum* with frozen bloodworms (Gamma, Chorleywood, UK). The fish were allowed to settle for a minimum of 48 h following capture before experimentation. Feeding was stopped on the day prior to the start of all experiments to maintain water quality and reduce fish stress.

Choice of anaesthetic and its effect on gyrodactylids transmission
To investigate this, 20 individual sticklebacks were terminally euthanised with 0.01 M 2-phenoxyethanol (MERCK-Germany) and placed in individual Petri dishes containing clean water at 10 °C. Dead hosts were observed under an Olympus SZ30 stereomicroscope and the time at which each gyrodactylid abandoned the fish recorded over 100 minutes. All the gyrodactylids migrating off the fish were collected with a 200 µL pipette, transferred to slides and were mounted under a coverslip with a drop of Malmberg's fixative (ammonium picrate glycerine). After 100 min, the experiment was terminated and the population of gyrodactylids leaving the fish were staged. Twenty fish were euthanised using a needle and piercing the head once destroying the brain and placed in individual Petri dishes containing clean water at 10 °C following the same procedure for the anaesthetic experiment. Parasites used in this study sat stationary on the substrate of the Petri dish and making occasional circular exploratory movements. No moribund or dead parasites were considered during the experiments. The maturity status of worms was recorded using a compound microscope (Olympus BX51) under a ×100 / oil immersion objective. The fish carcasses containing non-migrated parasites were fixed in 80 % ethanol for assessment of the maturity status of immigrated individuals to establish the overall population structure. Several features of the recovered parasites were assessed: i) an identification of species using the hard parts of the opisthaptor; ii) four developmental states were recognised to describe the stage of maturation of each parasite; no daughter and no male copulatory organ (MCO) (ND / N MCO); 2) no MCO and a daughter present *in utero* (N MCO / D); 3) MCO present but no daughter (MCO / ND); and, 4) both MCO present and a daughter present (MCO / D). A daughter/embryo was considered to be present if the rudimentary hard parts of the opisthaptor or attachment hooks were evident. The MCO was considered to be present only if the spines were clearly visible.

Results

From 40 trials (n = 20 anaesthetic, n = 20 pitting), a total of 544 gyrodactylids were recorded and staged. The results showed that when hosts were euthanised using the anaesthetic 2-phenoxyethanol, then a total of 47 parasites were found to have transferred to the bottom of the Petri dish. Of these, 8 ± 0.82 (*i.e.* 17 %) parasites had an MCO and a daughter present; 14 ± 0.73 (*i.e.* 30 %) had no MCO but a daughter present; 23 ± 1.26 (*i.e.* 49 %) had an MCO and daughter absent, and finally, 2 ± 0.30 (*i.e.* 4 %) had no MCO and no daughter present.
Examination of the 198 worms that remained on the parasitised fish host were made up of 42 ± 1.65 (*i.e.* 21 %) parasites that had an MCO and a daughter present; 108 ± 3.0 (*i.e.* 54 %) parasites that had no MCO but a daughter present; 36 ± 2.01 (*i.e.* 18 %) that had an MCO but no daughter, and finally, 12 ± 6.06 (*i.e.* 6 %) parasites that had neither an MCO or a daughter present (Table 1). A Wilcoxon (non-parametric) test was used to test whether there was a difference in the number of parasites transferring to new hosts from infected hosts killed by one of two methods (*i.e.* pithing *versus* anaesthetic) ($W = 157$; $p = 0.245$); the results were not significant. The mean number of parasites transferring from hosts killed with anaesthetic were 2.35 ± 2.23 (mean ± S.D.) whilst those from hosts killed by pithing were 3.05 ± 2.21 (Fig. 1).

Pithing hosts and its effect on gyrodactylid transmission
The results showed when hosts were pithed a total of 61 parasites transferred from the hosts to the bottom of the Petri dish. Of these, 15 ± 1.20 (*i.e.* 25 %) had an MCO and a daughter present; 12 ± 0.82 (*i.e.* 20 %) lacked a MCO but had a daughter present; 22 ±

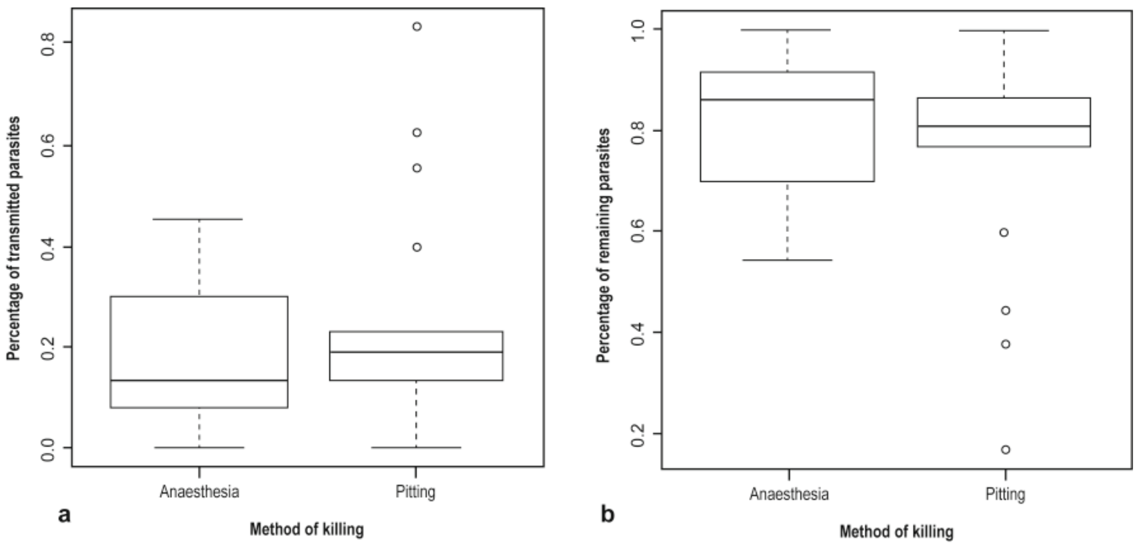

Fig. 1. a) Box plot of the proportion of gyrodactylids transferring from fish killed by an overdose of anaesthetic and from those killed by pithing.
b) Proportion of gyrodactylids remaining on fish having been killed by an overdose of anaesthetic and on those killed by pithing

1.29 (*i.e.* 36 %) had an MCO but a daughter absent, and finally, 12 ± 0.68 (*i.e.* 20 %) parasites had either an MCO or a daughter present (Table 1). Of the 238 worms that remained on the host killed by pithing 64 ± 3.57 (*i.e.* 27 %) parasites had an MCO and a daughter present; 115 ± 5.0 (*i.e.* 48 %) parasites had no MCO but a daughter present; 36 ± 2.04 (*i.e.* 15 %) had an MCO but no daughter, and finally, 23 ± 1.46 (*i.e.* 10 %) parasites lacked both an MCO and a daughter (Table 1).

Discussion

This study describes aspects of the transmission of *Gyrodactylus gasterostei* to its fish host, including the role of maturity and reproductive status in migration and the possible effect of anaesthetic. In the dead host migration in both experiments, the results suggested that worms with the presence of a developed MCO were more likely to leave the host (Table 1). The population remaining on the host was largely immature. This suggests that gyrodactylids that have given birth at least once are more likely to leave the host following host death in order to colonise new hosts, as a MCO appears after gyrodactylids have given birth for the first time (at an age of 24 – 30 h at 13 °C for *G. gasterostei* according to Harris, 1985a). Grano-Maldonado (2014b), showed a significantly higher probability of parasites transferring in the groups with an MCO (penis) than those lacking an MCO, being a key factor during oral route transmission from dead to live fish during scavenging activities. Young flukes, which have not given birth, have high life expectancy, and may remain on the fish until after they have given birth in order to maximise population numbers. Transmission from a dead host appears to be more efficient than from living ones. Olstad *et al.* (2006) concluded that parasites that remained on a dead host survived and maintained their infectivity for longer periods than detached worms.

During this study, no other gyrodactylid species (i.e *G. arcuatus*) were found on the surface of the sticklebacks by confirming the absence of excretory bladders, and the shape of the ventral bar (Harris 1985b). This observation may explain why stickleback differed in natural infection levels of *Gyrodactylus arcuatus* (de Roij *et al.,* 2011). The decision to leave a fish may also reflect the nutritional status of the worm, these perhaps requiring a filled gut or high stored reserves before leaving the host. Cable *et al.* (2002) noted that detached, starved parasites can abort their offspring (embryos) and that an interruption in nutrient flow to the embryo might have a significant impact on the reproductive rate. A significant percentage of worms in the anaesthetic trial detached sooner during a transfer opportunity or accidentally dislodged, appear to lose their embryos subsequently [Observation data]. Of the daughters that were lost, some were quite advanced in their development *i.e.* had fully developed marginal hooks and nearly complete hamuli but had underdeveloped hamuli roots and dorsal / ventral bars. This represents an interesting observation because if worms can be forced to transfer off their hosts prematurely and this results in an increased chance of embryo loss, then this could have a significant effect on the number of worms surviving and managing to reattach. This research aimed to evaluate the use of anaesthetic concerning transmission and its possible effect on the parasite's transmission, since the effect of some compounds i.e octopaminergic substances has been studied previously on *Gyrodactylus* affecting the parasite's ability to locate and remain on its host (Brooker *et al*., 2011). In our best knowledge, the effect on the use of anaesthetic and the outcome of this research hasn't been ever evaluated previously. This study indicates that the use of the anaesthetic 2-phenoxyethanol does not affect the population of gyrodactylids which transmit off the host.

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References

Bakke, T.A., Cable J., Harris, P.D. (2007): The biology of gyrodactylid monogeneans: the "Russian Doll-killers". *Adv. Parasitol.,* 64: 161 – 376. DOI:10.1016/S0065-308X(06)64003-7

Brooker, A.J., Grano-Maldonado, M.I., Irving, S., Bron, J., Longshaw M., Shinn, A.P. (2011): The effect of octopaminergic compounds on the behaviour and transmission of *Gyrodactylus. Parasite Vectors*, 4: 207. DOI: 10.1186/1756-3305-4-207

Cable, J., Harris, P.D. (2002): Gyrodactylids developmental biology historical review, current status and future trends. *Int. J. Parasitol.*, 32: 255 – 280. DOI: 10.1016/S0020-7519(01)00330-7

De Roij, J., Harris P., MacColl, A. (2011): Divergent resistance to a monogenean flatworm among three-spined stickleback populations. *Func. Ecol.,* 25: 211 – 217. DOI: 10.1111/j.1365-2435.2010.01775.x

Grano-Maldonado, M.I. (2011a). *The biological and behavioural basis of host selection in transmission of Gyrodactylus (Monogenea).* PhD Thesis, Stirling Univeristy, UK.

Grano-Maldonado, M., Bron, J., Longshaw, M., Shinn, A. (2011b): The accidental transfer of *Gyrodactylus* (Monogenea) during short duration fish transportation. *Fish Pathol.,* 46 (3): 71 – 79. DOI: 10.3147/jsfp.46.71

Grano-Maldonado, M.I. (2014a): Ultrastructure of the external sensory apparatus of *Gyrodactylus gasterostei* Gläser, 1974. *Microsc. Res. Tech.*, DOI 10.1002/jemt.22396

Grano-Maldonado M. (2014b): *Gyrodactylus gasterostei* a difficult meal to swallow for the three-spined sticklebacks, *Gasterosteus aculeatus* L. *Scanning,* DOI:10.1002/sca.21162

Harris, P.D. (1985a): Observations on the development of the male reproductive system in *Gyrodactylus gasterostei* Gläser, 1974 (Monogenea, Gyrodactylidae). *Parasitology,* 91: 519 – 529. DOI: 10.1017/S0031182000062764

Harris, P.D. (1985b): Species of *Gyrodactylus* von Nordmann, 1832 (Monogenea: Gyrodactylidae) from freshwater fishes in southern England, with a description of *Gyrodactylus rogatensis* sp. nov. from the bullhead *Cottus gobio* L. *J. Nat. Hist.,* 19: 791 – 809. DOI: 10.1080/00222938500770491

Harris, P.D. (1993): Interactions between reproduction and population biology in gyrodactylid monogeneans – A review. *Bull Fr. Peche Piscic.*, 1: 47 – 65. DOI: 10.1051/kmae:1993011

Olstad, K., Cable, J., Robertsen, G., Bakke, T. A. (2006): Unpredicted transmission strategy of *Gyrodactylus salaris* (Monogenea: Gyrodactylidae): survival and infectivity of parasites on dead hosts. *Parasitology*, 133: 33 – 41. DOI: 10.1017/S0031182006009966