Monogenean Parasites of Non-Native Freshwater Fish in New Zealand: Ecology and New Distributional Records

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ETHAN D. RENNER

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Abstract

Monogeneans are common aquatic ectoparasites within the phylum Platyhelminthes. Freshwater monogeneans have been little studied in New Zealand, with only three taxa having been recognised in the country until recently, including only two to species level. Twenty-one non-native freshwater fish species have established populations in New Zealand, yet only one of these has been reported with monogenean infestations. Based on the diversity of monogenean species known from these fish species globally, it is likely that many monogenean species will have established populations in New Zealand that remain unreported. The prevalence and intensity of parasite infection and infestation are known to vary with season and in relation to host factors such as size and sex, but little is known of their ecology in New Zealand freshwater fish species.

I report for the first time the presence of eleven monogenean species infesting non-native freshwater fish in New Zealand. Two of these species belong to the family Ancyrocephalidae, a family new to New Zealand. A seasonal study of two species infesting *Gambusia affinis* (Baird & Girard, 1853) in two interconnected ponds in Hamilton, *Salsuginus seculus* (Mizelle & Arcadi, 1945) and *Gyrodactylus gambusiae* Rogers and Wellborn, 1965, represents the first ecological study of monogeneans undertaken in New Zealand, a country with a mild climate and narrow annual temperature range relative to better studied northern temperate populations. The prevalence of *S. seculus* changed significantly over the course of a year, reaching a peak during the summer. However, no significant correlation with temperature was found for either the prevalence or mean intensity of *S. seculus*. Prevalence was instead correlated with the concentration of chlorophyll *a* and pH. However, it is likely these correlations were simply due to chlorophyll *a* concentrations similarly increasing over summer affecting the pH, rather than either variable being causative. As *G. affinis* is known to experience large increases and decreases in population density annually, with individuals coming into physical contact during breeding, this was likely the cause of the increase in monogenean prevalence. A greater number of hosts would provide more habitat for monogeneans and their increased density would facilitate transmission of the parasites. The sex of fish was found to be a significant predictor of prevalence and intensity of monogeneans *S. seculus*, but this was on the basis of size. Longer and heavier fish had higher prevalence and mean intensity of *S. seculus*, and as female *G. affinis* are typically larger than males, this led to the difference in infestation between the sexes. Larger individuals represent larger habitat patches and for this may be the reason that prevalence and intensity of monogeneans was higher on these individuals. A similar test was conducted on *Carassius auratus* (L.), where two size classes were observed, representing juveniles and adults. The juvenile fish had significantly higher prevalence and intensities of *Dactylogyrus* spp., indicating a parasite vulnerability in juveniles. This thesis contributes to the knowledge of the species diversity and ecology of freshwater monogeneans in New Zealand.

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Introduction

Many of the metazoan phyla have members that utilise parasitism as a feeding method (Poulin, 2007; Weinstein & Kuris, 2016). Platyhelminthes represents a phylum of which many members are parasites, notably the classes, Digenea (flukes), Cestoda (tapeworms) and Monogenea (Poulin, 2007). The monogeneans are distinguished from the other classes of parasitic platyhelminths by their direct lifecycle (which involves only a single host) and their morphology (Whittington & Chisholm, 2008; Goater et al., 2014). They are generally ectoparasitic unlike the endoparasites digeneans and cestodes, attaching themselves to their hosts by means of a unique organ on their posterior known as the haptor, and secondarily by the use of the anterior prohaptor (Whittington & Chisholm, 2008; Goater et al., 2014). The haptor is equipped with anchors known as hamuli in the subclass Monopisthocotylea or clamps or suckers in the subclass Polypisthocotylea (Whittington & Chisholm, 2008). Monogeneans are typically parasites of marine and freshwater fish, attaching themselves to the body surface and fins as well as to the gill filaments (Whittington & Chisholm, 2008) However, they are also known to be endoparasites of fish (Llewellyn, 1960) and ectoparasites of other groups of animals such as anurans (Tinsley & Earle, 1983) and testudines (Pichelin, 1995). One species, *Oculotrema hippopotami*, is even known to attach to the eye of the hippopotamus (Stunkard, 1924).

The lifecycle of monogeneans is a simple one compared to many other parasites; they have no intermediate hosts, and are in fact, highly host specific, with the majority of monogenean species infesting only a single host species each (Poulin, 1992; Whittington & Chisholm, 2008). Monogeneans are hermaphroditic and may self-fertilise but typically cross-fertilise with other individuals (Whittington & Chisholm, 2008). Fertilised eggs are released into the water column where they may occasionally attach to some substrate (Chubb, 1977; Whittington & Chisholm, 2008). From these eggs emerge free-swimming ciliated larvae known as oncomiracidia, which swim to their host and attaching themselves; here, the monogeneans will remain for the rest of their lives (Chubb, 1977; Whittington & Chisholm, 2008). The exception to this form of reproduction and transmission are members of the family, Gyrodactylidae (Bakke et al., 1992; Bakke et al., 2007). Some of these gyrodactylids are not oviparous like the rest of the monogeneans but are instead viviparous, even considered hyperviviparous (Bakke et al., 2007). Fully grown daughters, are retained, in utero, until they contain their own embryos. The young are born fully grown and able to give birth themselves shortly after (Bakke et al., 2007).

Monogeneans do not necessarily cause disease in their hosts but they may be important pathogens. The subclass Monopisthocotylea possess haptoral anchors known as hamuli that may damage their host. For example, in the case of gill tissue, the hamuli are inserted into the lamella, which may cause hyperplasia, an increase in mucus production, and fish mortality, especially in captivity (Chisholm and Whittington, 1998; Kritsky & Heckmann, 2002; Igeh & Avenant-Oldewage, 2019). Monogeneans may spread through host populations in outbreaks. The most notable of these is the *Gyrodactylus salaris* epidemic in Norway, which caused major reductions in populations of wild and farmed salmon (Bakke et al., 2007).

New Zealand is home to 63 native freshwater fish species and 21 introduced freshwater fish species, 19 of which are established (Duggan & Collier, 2018). Many of these fish are ecologically important as they negatively impact freshwater ecosystems (Rowe, 2007). These fish, particularly *Cyrpinus rubrofuscus* Lacépède, 1803. and *Carassius auratus* (L.), are host species for several monogenean species elsewhere (Hoffman, 1999). Despite this, only two monogenean species have been observed from non-native freshwater fish species in New Zealand (Hewitt & Hine, 1972; Edwards & Hine, 1974). *Dactylogyrus ctenopharyngodonis* Achmerow, 1952, and *Gyrodactylus ctenopharyngodontis* Ling, 1962, were found on the gills and the fins and scales of *Ctenopharyngodon idella* (Valenciennes in Cuvier & Valenciennes, 1844), respectively. These were observed on *C. idella* fry in a consignment from Hong Kong, which were treated for the monogenean infestation and the parasites were apparently eliminated (Edwards & Hine, 1974). Whether these monogeneans may be found on wild *C. idella* in New Zealand is unknown. The only other monogenean known from New Zealand freshwater fish, native or non-native, is an unknown *Gyrodactylus* sp. from the gills of the native *Galaxias brevipinnis* Günther, 1866, (Hewitt & Hine, 1972).

Seasonal changes in monogenean populations have been studied on numerous occasions across the globe (Chubb, 1977; Valtonen et al., 1990; Mo, 1992; Gutierrez, 1994; Ozer & Erdem, 1999; Davidova, 2005; Aydogdu, 2006; Blažek et al., 2008; Poulin, 2020; Wenxiang et al., 2022; Mo et al., 2023). Often, studies of monogenean population changes conclude temperature to be an important factor. However, a meta-analysis by Poulin (2020) found that this was not the case. Freshwater monogenean species tend to increase in abundance during the summer months in temperate regions, but populations do not change significantly between the wet and dry seasons in the tropics (Ozer & Erdem, 1999; Aydogdu, 2006; Poulin, 2020). Other monogenean species experience population growth in colder months (Valtonen et al., 1990; Wenxiang et al., 2022), still others show multiple instances of population increase and decrease annually (Chubb, 1977; Wenxiang et al., 2022). Seasonal population studies of freshwater monogeneans have mostly been undertaken in continental regions, such as the Czech Republic (Dávidová et al. 2005; Blažek 2008), Finland (Valtonen et al. 1990), Poland (Prost 1963; Wierzbicka 1974), Turkey (Ozer and Erdem 1999; Aydogdu 2006), and the USA (Crane and Mizelle 1968; Rawson and Rogers 1972). These are temperate continental locations and experience wide ranges in temperature throughout the year (Duckson, 1987). New Zealand differs climatically from these places as an isolated set of islands which do not experience temperature extremes like in continental regions (Green et al., 1987). Compared to lakes at similar latitudes, New Zealand lakes experience a narrower range of annual temperature changes. (Green et al., 1987). No seasonal studies of freshwater monogeneans have been undertaken in New Zealand previously and the unique climatic conditions in New Zealand make it a particularly good place in which to examine freshwater monogenean changes.

Larger fish species have been proposed as being more likely to host ectoparasites and as harbouring more parasites but the evidence for this is inconsistent (Kuris et al., 1980; Rohde et al., 1995; Poulin, 2000; Rubio-Godoy, 2008; Madanire-Moyo et al., 2011). On the basis of size alone, it has been suggested that larger fish present more favourable patches of habitat as resources are in greater supply (Kuris et al., 1980). Larger fish are also older, and thus, have had more time to accumulate parasites. Infestation and infection by parasites in terms of host age is not clear cut, however, and younger individuals may have more parasites than their older counterparts (Wunderlich et al. 2022). Sex has also been important in the infection and infestation of host animals by parasites, especially for displaying males (Hamilton & Zuk, 1982; Folstad & Karter, 1992). Testosterone has been shown to suppress immune function (Foo et al. 2016, Roved et al. 2017) and male teleosts have higher testosterone levels than females (Borg 1994). This gives credence to the possibility that more male fish will be infested with monogeneans and they may have higher intensities of infestation.

In chapter one of this thesis, I examined *Gambusia affinis* individuals and for the first time in New Zealand, observed the presence of *Salsuginus seculus* (Mizelle and Arcadi, 1945) and *Gyrodactylus gambusiae* Rogers and Welborn, 1965, infesting *Gambusia affinis* (Baird and Girard, 1853). The discovery of these parasites is discussed in terms of enemy-release as *Gambusia affinis* in New Zealand originating from a Hawaiian population, itself sampled from a Texan population (McDowall, 1990), indicates that monogeneans are unlikely to be lost during species introductions. With this in mind, it is expected that many more monogeneans should be found on non-native freshwater fish in New Zealand.

In chapter two, the aims were to examine the factors influencing monogenean populations across and year and the host factors that affected levels of prevalence and intensity. A population of *S. seculus* infesting *G. affinis* in a freshwater system on the Hamilton campus of the University of Waikato were studied. The prevalence and intensity of *S. seculus* were recorded on a monthly basis over the course of a year, also making note of *Gyrodactylus gambusiae* individuals infesting *Gambusia affinis*. Measurements of the environmental variables, temperature, pH, dissolved oxygen, and chlorophyll *a* concentration were made to determine if a correlation existed between these and the parasitological indices. Fish sampled were measured, weighed, and sexed to test the hypothesis that these host factors may result in variation in monogenean infestation.

In chapter 3, on the basis of the prediction of the first chapter, a survey would be conducted on several non-native freshwater fish species. As a part of this survey, the effect of host age on monogenean infestation in *C. auratus* would be examined.

These chapters were written as manuscripts for publication and as such, some of the introductory material is repeated among chapters.

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Chapter One

Salsuginus seculus (Monogenea: Ancyrocephalidae) and *Gyrodactylus gambusiae* (Monogenea: Gyrodactylidae) Newly Recorded Infesting *Gambusia affinis* (Teleostei: Poeciliidae) from New Zealand

Abstract

The North American western mosquitofish, *Gambusia affinis* (Baird and Girard, 1853), has been introduced into a number of countries around the world, primarily for the control of mosquito populations. *Gambusia affinis* is known to harbour several monogenean parasites, which have the potential to be co-introduced to new regions. However, despite the broad non-native distribution of this fish species, their monogenean parasites have been reported from a relatively small number of locations. Parasitological investigations have identified very few freshwater monogenean parasites from freshwater fish in New Zealand. Two monogenean parasites were recorded infesting *G. affinis* in New Zealand; *Salsuginus seculus* (Mizelle and Arcadi, 1945) (a new genus to New Zealand) on the gills and *Gyrodactlyus gambusiae* Rogers and Welborn, 1965, on the gills and surface of the fish. Despite the broad distribution of *G. affinis*, *S. seculus* and *G. gambusiae* have been reported in few countries due, perhaps, to lack of research effort on monogeneans, their small size, enemy-release, or a combination of these factors. Based on the diversity of monogeneans in other countries that are found associated with fish with non-native populations in New Zealand, it is likely that many unrecognised non-native monogeneans are established in New Zealand's freshwater ecosystems alongside their fish hosts. Further, the number of introduced monogeneans is likely to be even higher than is currently appreciated when considering non-native fish in the aquarium trade and other introduced freshwater fauna, such as terrapins.

Introduction

The western mosquitofish, *Gambusia affinis*, has spread globally from its native distribution in south-eastern USA and northern Mexico (Ling, 2004; Pyke, 2008). In attempts to control mosquitoes it has been introduced into other regions of the USA and numerous other countries

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(Seale, 1917; Krumholz, 1948; Ling, 2004; Jourdan et al., 2021). For example, following initial unsuccessful introductions, *G. affinis* sourced from non-indigenous populations in Hawaii was established in New Zealand at the Auckland Botanical Gardens in 1930 (now known as the Auckland Domain; McDowall (1990)). Despite initial confidence in the ability of *G. affinis* to control mosquitoes (Washino, 1969), subsequent research has contested the degree of its efficacy (Kramer et al., 1987), and the species is now widely regarded as a harmful invader in many of the ecosystems to which it has been introduced (Rupp, 1996; Pyke, 2008; Tsang & Dudgeon, 2021).

The global spread of non-indigenous fishes provides the opportunity for their parasites to be dispersed with them and thus, expand their distributions. This is illustrated by the establishment of various parasite species with their non-native fish hosts in Hawaii (Font, 2003). Similarly, the introduction of grass carp, *Ctenopharyngodon idella* (Cuvier & Valenciennes, 1844), into New Zealand required treatment to remove parasites following their post-border detection (Edwards & Hine, 1974).

Monogenea is a diverse class of Platyhelminthes most commonly found infesting the surfaces of fish, including the gills and fins, and sometimes, but not always, causing disease (Whittington & Chisholm, 2008). Most monogeneans are oixenous; that is, they have an obligate relationship with a single host species (Whittington & Chisholm 2008). One monogenean species, *Salsuginus seculus*, infesting the gills of *G. affinis* is a member of the family Ancyrocephalidae. Since the initial description of *S. seculus* in 1945, there have been numerous new distribution records of this species. It has been recorded from *G. affinis* in various states in the USA, including Texas (Nowlin et al., 1967; Meade & Bedinger, 1972), California (Mizelle & Arcadi, 1945), Oklahoma, Kansas, Arkansas (McAllister et al., 2015), Louisiana (Duobinis-Gray & Corkum, 1985), and Hawaii (Font, 1997), with the latter being the origin of the New Zealand *G. affinis* populations. Outside of the USA, *S. seculus* has been reported from the Bahamas (Hanek & Fernando, 1972), though this has been contested due to the absence of *G. affinis* there, with the related *G. manni* Hubbs, 1927 and *G. puncticulata* Poey, 1854 occurring there instead (Nitta & Nagasawa, 2014). It has also been reported in Japan by Nitta and Nagasawa (2014), who noted that in spite of parasitological investigation of *G. affinis* in New Zealand, *S. seculus* was yet to be located in that country. A second species known to infest *G. affinis* is a member of the Gyrodactylidae family, *Gyrodactylus gambusiae*. First observed in 1965 in Florida, little literature exists for this species (Rogers & Welborn, 1965; Rubio-Godoy et al., 2010). Distribution records are limited for both species despite the global range of *G. affinis*. Here, new distributional records of *S. seculus* and *G. gambusiae*, both infesting *G. affinis* in New Zealand, are reported.

Materials and methods

From April 2022 to March 2023, *G. affinis* were collected from Knighton and Oranga Lake, two interconnected ponds on the University of Waikato campus, Hamilton (37°47′08.7″S; 175°18′53.3″E and 37°47′12.4″S; 175°18′57.2″E, respectively). Fish sampling was carried out by placing minnow traps around the edges of the ponds and retrieving them approximately 24 hours later. Upon collection, *G. affinis* were transferred to the laboratory and examined immediately. For each location, the two lakes and the stream connecting them, 50 fish specimens were examined for parasites. Hand netting was also utilised to capture more fish if the traps had failed to capture sufficient fish to meet an ideal minimum of 50 individuals to examine. Fish were humanely euthanised using an anaesthetic overdose of benzocaine and promptly examined for parasites to ensure that fresh material was being observed. Following examination of the outer surface of each fish, the gills were extracted using forceps and examined using an Olympus SZ40 dissecting microscope. When a parasite was observed, it was removed from the body or gills using fine needles. The live parasite, immersed in water, had a coverslip placed on it and was observed for photography and morphometric identification using a DMRE Leica microscope. Photographs and measurements were taken of monogeneans infesting *G. affinis* on 29 and 31 August, 28 September, 5 October 2022, and 7 January, 25 and 30 March 2023. By examining freshly euthanised fish, parasites were located on account of their movement. Further, examining live parasites helped to mitigate contraction of their bodies as may happen when specimens are fixed in alcohol (Nitta & Nagasawa, 2014). For *S. seculus*, measurements were taken of the length and width of the body, the haptor size, and structures of the haptor, including the hamuli (total length, deep root length, superficial root length, blade length), as well as the penis and accessory piece. Additionally, the number of marginal hooks was recorded. These measurements were recorded according to Murith and Beverly-Burton (1985), who set out a process for measuring sclerotised structures of *Salsuginus* species. These measurements were compared with the species' original description (Mizelle & Arcadi, 1945) and a subsequent distributional record in Japan (Nitta & Nagasawa, 2014) to confirm the species' identity. Measurements for *G. gambusiae*, and confirmation of the species, were made following the species' description in Rogers and Welborn (1965). Measurements for *G. gambusiae* included the length and width of the body as well as the haptor size, hamuli lengths (root lengths were not taken as this species does not have structures like those of *S. seculus*), bar widths and lengths, and number of marginal hooks. Measurements were taken for those structures that were clearly visible without chemical staining of the specimen, and which are the most relevant for the identification of monogeneans.

Results

Salsuginus seculus

Salsuginus seculus were found on the gills of *G. affinis* captured at all sites and on all dates when measurements were taken as listed prior, except for 28 September (Figure 1). In total, 551 specimens of *S. seculus* were observed from *G. affinis* from April 2022 to March 2023. For the dates in which measurements were taken, the numbers of observed *S. seculus* individuals are as follows: August, 6; October, 8; January, 21; March, 29. Of these, 14 were measured. All measurements are given in micrometres and expressed as the mean ± the standard deviation, with the range and number, *n*, given. Body elongate, 238.8 ± 42.3 (156–286; *n* = 10) long and 118.1 ± 32.8 (65–173; *n* = 11) wide. Specimens generally flattened under coverslip pressure. Total length of haptor, 38.0 ± 8.4 (30–54; *n* = 13). Total length of hamuli, 17.7 ± 2.4 (14–23; *n* = 14). Length to notch, 16.4 ± 1.7 (14–19; *n* = 14). Deep root length, 2.3 ± 0.7 (1.5–4.0; *n* = 14). Length of superficial root, 4.9 ± 1.6 (2–8; *n* = 14). Blade, 4.2 ± 0.9 (1.5–5.0; *n* = 14) long. Ventral bar, 21.5 ± 3.0 (19–26; *n* = 14) long, 2.5 ± 1.1 (1.0–3.5; *n* = 14) wide. Dorsal bar, 22.7 ± 2.8 (18.8–27.0; *n* = 14) long and 2.5 ± 0.6 (1.5–3.2; *n* = 14) wide. Length of penis, 12.3 ± 1.9 (11–15; *n* = 4). Length of accessory piece 12.8 ± 1.0 (12–14; *n* = 4). Specimens had 7 pairs of marginal hooks. All specimens had one pair of eyespots, with some possessing a second less pronounced pair, though in most cases these appeared reduced to dispersed melanistic granules.

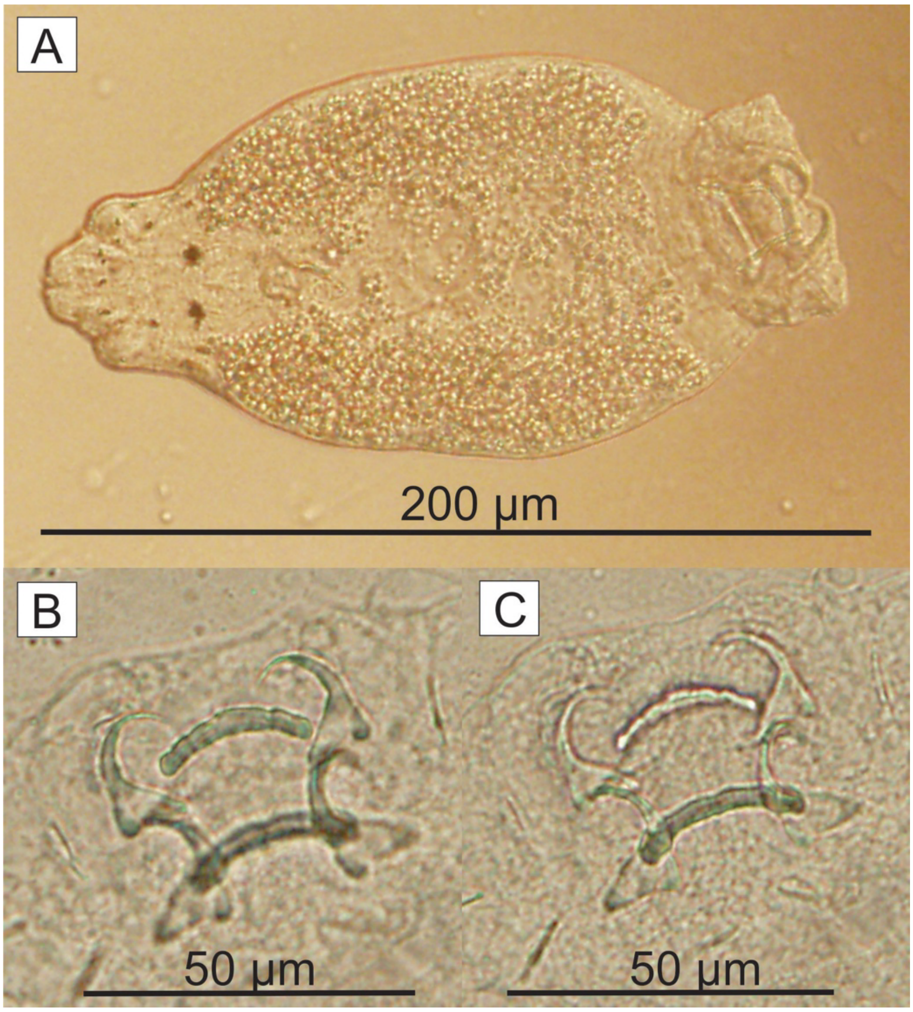

Figure 1. Photographs of *Salsuginus seculus*: (A) whole body (400x), and (B, C) haptor, on two planes of focus (1000x). Photograph by E.D. Renner.

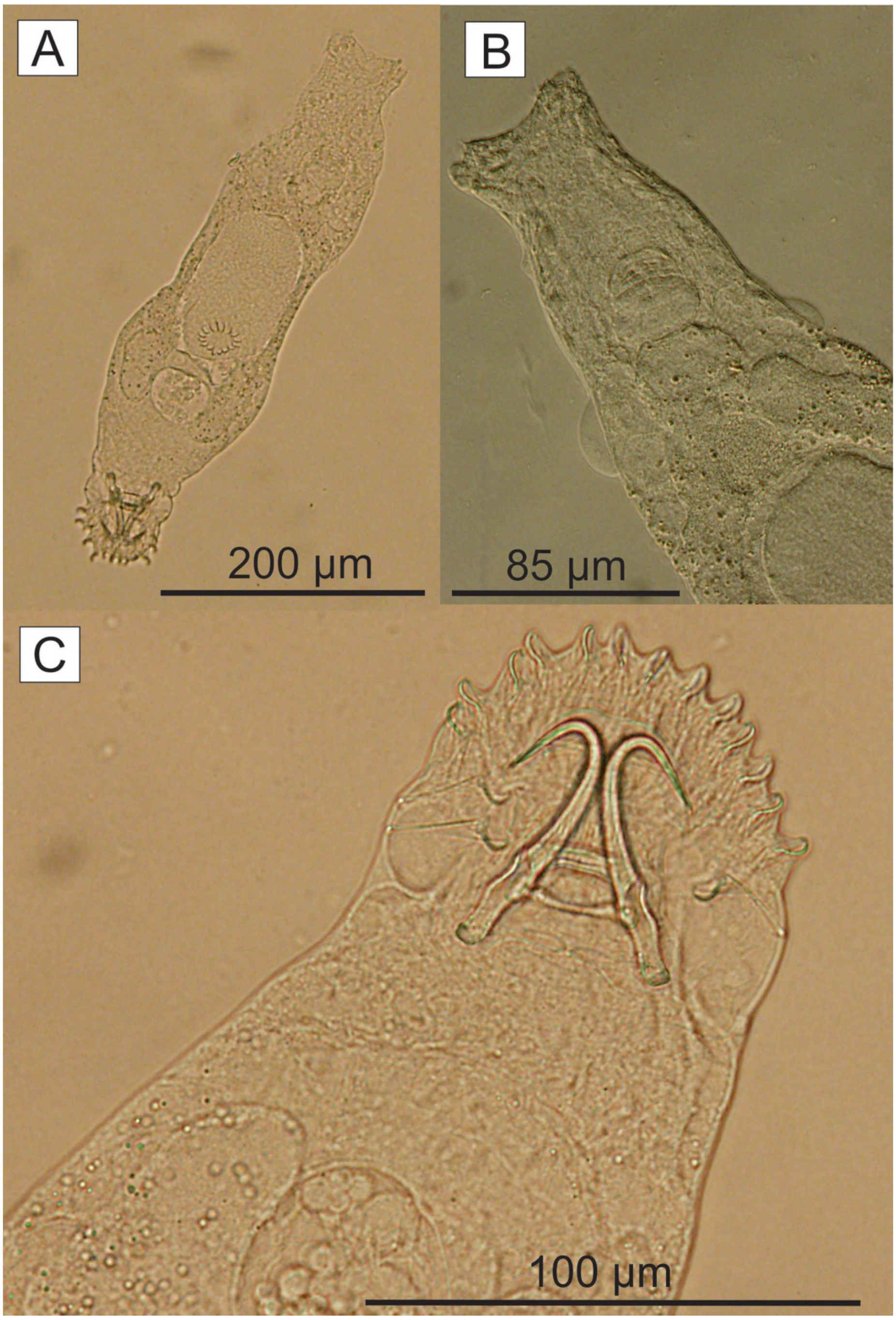

Figure 2. Photographs of *Gyrodactylus gambusiae*: (A) Whole body (200x), (B) anterior (400x), and (C) haptor (400x). Photograph by ED Renner.

Gyrodactylus gambusiae

One specimen of *G. gambusiae* was observed on 28 September 2022, four on 7 January 2023 and 5 on 25 and 30 March (Figure 2). Specimens were found to occur on both the gills and the fins. Measurements were made for one specimen in September 2022, one from January, and two in March 2012 (*n* = 4). All measurements are given in micrometres and expressed as the mean of measurements ± the standard deviation, with the range given. Elongate body with major bifurcation at anterior end, body length, 345.5 ± 77.5 (262–428), width 78.3 ± 16.7 (58–96). Umbrella shaped haptor, 62.3 ± 6.3 long (55–68). Hamuli length, 49.5 ± 1.3 (48–51). Ventral bar and shield, 18.5 ± 5.3 long (14–23) and 15.3 ± 1.3 wide (14–17). Dorsal bar, 20 ± 0.8 long (19–21), 1.6 ± 0.5 wide (1–2). 16 marginal hooks were observed.

Discussion

The morphologies of the two parasite species reported here were overall consistent with the descriptions reported in the literature for *S. seculus* (Mizelle & Arcadi, 1945; Nitta & Nagasawa, 2014) and *G. gambusiae* (Rogers & Welborn, 1965). Further, being found to infest *G. affinis* is also a key diagnostic feature that confirms the identity of these species; as monogeneans are highly host specific (Poulin, 1992; Whittington et al., 2000), identification of the host species aids their identification greatly. Of particular note, *G. gambusiae* specimens were found not only on the fins, but also on the gills of *G. affinis*. Rogers and Welborn (1965) reported this species to infest the fins and body of the fish only. However, examination of the gills was not mentioned in their methodology. Since their study, no literature has been published to note the greater range of attachment on *G. affinis* and as such these observations are the first to note the occurrence of this monogenean on the gills. That so few *G. gambusiae* were observed is also noteworthy. Why *G. gambusiae* has low prevalence in the current study may be due to seasonal changes in abundance, with sampling not coinciding with peak infestation. Perhaps more likely, low infestation rates may be due to this species occurring at low frequencies on *G. affinis* in general. Studies on the populations of other gyrodactylid species have found greater numbers and higher infestation prevalence than seen here (e.g., Scott & Anderson, 1984; Mo, 1992). The literature on *G. gambusiae*, in particular, is not extensive, not extending greatly beyond its initial description, with no studies existing on their prevalence, intensity, or population dynamics. This may indicate that their prevalence and abundance are low globally, and as such, they are rarely sighted.

These new distributional records indicate that *S. seculus* and *G. gambusiae* have been previously overlooked in New Zealand. It is likely that these parasites accompanied *G. affinis* when it established in the Auckland Botanic Gardens in 1930, from mainland USA via Hawaii. As such, they have likely existed there for some 90 years. Nitta and Nagasawa (2014) noted that in spite of intense parasitological investigation of *G. affinis* in New Zealand by Zhang (2012), *S. seculus* had not been found there. Indeed, the investigation conducted by Zhang (2012) found no parasites on or in *G. affinis* at all. However, only a small subset of those collected were dissected and their gills inspected by this author, with the rest digested with an HCl and pepsin solution to search for internal parasites. Additionally, the bulk of sampling in that study was undertaken over the winter months, when parasite numbers might have been reduced. This makes it likely that these monogeneans would have been missed by that author. The finding of these parasites leaves the question as to how many more non-indigenous monogenean species are yet to be found in New Zealand. To date, around 5000 monogenean species have been described globally; however, the number of species overall may exceed 25000 (Kuchta et al., 2020). As of 2009, only 25 monogenean species had been recognised in New Zealand, including only three from freshwater fishes (Hine et al., 2000); the rest were all from marine fish species (Johns et al., 2009). *Ctenopharyngodon idella* possessed two of these freshwater monogenean species (*Dactylogyrus ctenopharyngodonis* Achmerow, 1952 and *Gyrodactylus ctenopharyngodontis* Ling, 1962), and was the only non-native freshwater fish reported to host monogeneans (Hine et al., 2000). Prior to release in New Zealand, grass carp were kept in tanks and were treated for parasites, which they were reportedly laden with. Once it was deemed that these infestations were under control, they were released into outdoor ponds (Edwards & Hine, 1974). However, subsequent research has not confirmed whether these parasites had been definitively eliminated. The third monogenean reported from freshwater fish in New Zealand was an undescribed *Gyrodactylus* species on the native kōaro, *Galaxias brevipinnis* Günther, 1866. There have been no apparent additions since this time. These facts demonstrate that Monogenea have been understudied in New Zealand, not just those from non-native fishes, but in general. There are 21 established non- indigenous freshwater fish species in New Zealand, each of which is likely to possess further unreported monogeneans. Parasites for a number of individual fish species are listed in “Parasites of North American Freshwater Fishes” by Hoffman (1999); from these lists, monogenean numbers for some non-native freshwater fish species living in New Zealand waters are as follows: 44 species of monogeneans from *Carassius auratus* (Linnaeus, 1758), 32 from *Cyprinus carpio* Linnaeus, 1758, 1 from *C. idella* (distinct from the 2 reported by Hine et al. (2000) from New Zealand), 4 from *G. affinis* (including the two reported here), 7 from *Ameiurus nebulosus* (Lesueur, 1819), 6 from *Poecilia reticulata* (Peters, 1859), 9 from *Onocorhyncus mykiss* (Walbaum, 1792), and 6 from *Salmo trutta* (Linnaeus, 1758). Together, this totals 111 monogenean species found infesting these fish species. This represents a large number of monogenean species with the potential to have entered New Zealand along with their fish hosts, which have not been reported. Even more introduced monogeneans may yet to be reported in New Zealand when organisms in the freshwater aquarium trade are considered, as well as animals such as the red-eared terrapin, *Trachemys scripta elegan*s (Wied-Neuwied, 1839), which are kept as pets but are commonly released into the wild. In Japan, for example, red-eared terrapin are known to carry two introduced monogeneans (Oi et al., 2012). The establishment of non-native parasites in New Zealand freshwaters has been noted as an area that is understudied, and not just for monogeneans (Lane et al., 2022). Not only are there likely to be more monogenean species introduced along with their hosts, previously unrecorded in New Zealand, it is also likely to be the case for other geographic regions.

The number of countries *G. affinis* has been introduced to far exceeds the number of countries from which these monogenean parasites have been recorded. It is possible that monogeneans, in particular, have gone unnoticed on freshwater fish due in part to a lack of research effort on this taxonomic group. Nevertheless, the enemy-release hypothesis (Keane & Crawley, 2002) predicts that host species may be liberated from their parasites, and has been hypothesised as a reason why parasites have not been observed infesting *G. affinis* in New Zealand (Zhang, 2012). Indeed, loss of parasites in this manner may have prevented other species entering New Zealand, explaining the lack of records for those taxa. Enemy-release may be responsible for *G. affinis* in some countries not being infested with *S. seculus* and *G. gambusiae*. In New Zealand, however, this is not the case. The mosquitofish established in New Zealand were first introduced from Texas into Hawaii (Seale, 1917), and then subsequently the Hawaiian fish were introduced into New Zealand in 1930 (McDowall, 1990). This stepwise introduction would presumably increase the chances of the parasites being lost along the way, yet this has not happened for these species. *Gyrodactlyus gambusiae* being found in New Zealand indicates that this species should be found infesting *G. affinis* in Texas and Hawaii, where *G. affinis* introduced into New Zealand originated from. However, this species has not been reported from either of these areas. When it comes to establishing populations in new areas, monogeneans may have a great advantage over other parasite groups by virtue of their life cycle. Many other parasites require multiple hosts to complete their life-cycle, which would preclude them from establishing populations in new countries, unless it coincided with the introduction of multiple host animals. Monogeneans, on the other hand, can enter countries with their single host, which allows them to complete their life-cycles as normal. As such, both *S. seculus* and *G. gambusiae* will also most likely be found in many other countries where *G. affinis* exists. This fact also illustrates the importance of parasitological surveys, as it is evident that enemy-release may not apply equally to all parasites and their hosts. As monogeneans do not typically cause disease in their hosts (Whittington & Chisholm, 2008), it is unlikely that introduced host populations would see decreases in death rates, or increases in birth rates or population growth rates, on the basis of becoming free of these parasites.

Changes in climate and the establishment of non-native parasites are likely to have diverse ecological ramifications that may be revealed with more research conducted in this area. The record of *S. seculus* and *G. gambusiae* living on *G. affinis* in New Zealand, alongside estimates of a great many more unreported monogeneans, highlights a lack of attention to invaders in this taxonomic group. Further study in this area will no doubt highlight a much greater number of freshwater parasite species in New Zealand than are currently recognised in the scientific literature.

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Chapter Two

Season, Size, and Sex: Investigating the Factors Influencing Monogenean Prevalence and Intensity On *Gambusia affinis*

Abstract

A number of studies on the seasonal changes in populations of various monogenean species have been conducted, though typically these have been undertaken in continental regions that experience a wide range of annual temperatures. The seasonal changes in prevalence and intensity of *Salsuginus seculus* (Mizelle and Arcadi, 1945) and *Gyrodactylus gambusiae* Rogers and Welborn, 1965, infesting *Gambusia affinis* (Baird and Girard, 1853) were investigated. This represents the first examination of seasonality for these species specifically and the first seasonal study of any monogenean population in New Zealand, a temperate country with a mild oceanic climate. Further, the prevalence and intensity of *S. seculus* with regard to fish size and sex was also examined. The prevalence of *S. seculus* changed significantly over the course of the year, peaking in the summer. The concentration of chlorophyll *a* was significantly correlated to monogenean prevalence and pH had a lesser, but still significant, correlation. The water temperature was not correlated to either the prevalence or mean intensity of *S. seculus*. However, as *G. affinis* is known to experience large seasonal population increases and decreases, with a breeding season that coincides with the increase in monogenean prevalence, the close association of *G. affinis* individuals during breeding, population increase, and subsequent population decrease, were likely the main driving factors in monogenean population changes. In New Zealand's mild oceanic climate, it appears that biotic factors were more important than abiotic ones in determining temporal variability in *S. seculus* prevalence in this study. *Gyrodactylus gambusiae* prevalence and intensity was low throughout the year, and this species was not observed on many months. Female *G. affinis* had a significantly higher prevalence and mean intensity of *S. seculus* than their male counterparts. The large size of females was found to be the best predictor of monogenean prevalence and intensity, which could be for a number of reasons, including representing a higher quality habitat patch for potential parasites, or being older, and thus having had more time to accumulate parasites.

Introduction

Monogeneans are an ectoparasitic class of the Platyhelminthes that most commonly infest fish, attaching themselves to surfaces such as the gills, body, and fins, primarily by means of an organ called the haptor (Whittington & Chisholm, 2008). Such infestations may occasionally, though not typically, lead to disease (Whittington & Chisholm, 2008). Unlike other platyhelminth parasites, such as those in the Digenea, monogeneans have a single-host life cycle and typically any single monogenean species normally infests only a single host species (Poulin, 1992). With the exception of the viviparous members of the Gyrodactylidae family, which produce fully grown young able to transmit directly to new hosts via brief contact between fish (Tepox-Vivar et al., 2022), monogeneans release eggs directly into the water column that hatch into ciliated larval oncomiracidia. These oncomiracidia then swim to find and attach to a prospective host where they mature into adult parasites (Chubb, 1977; Whittington & Chisholm, 2008).

A number of studies exist on seasonal changes in monogenean populations globally (e.g. Chubb, 1977; Valtonen et al., 1990; Mo, 1992; Gutierrez, 1994; Ozer & Erdem, 1999; Davidova, 2005; Aydogdu, 2006; Blažek et al., 2008; Madanire-Moyo et al., 2011; Poulin, 2020; Li et al., 2022; Mo et al., 2023). Seasonal changes in parasitological indices such as prevalence and intensity have most commonly been associated with seasonal variability in water temperature. Often, freshwater monogenean species increase in abundance when water is warmer during the summer months in temperate regions (Ozer & Erdem, 1999; Aydogdu, 2006; Poulin, 2020). In the tropics, however, where temperature is more stable throughout the year, freshwater monogenean infestations do not appear to change significantly among seasons (Poulin, 2020). Despite this general trend, some monogenean species in temperate regions show greater prevalence and abundance in colder months (Valtonen et al., 1990; Li et al., 2022) while others show multiple instances of high prevalence and intensity throughout the year (Chubb, 1977; Li et al., 2022). It is possible that variability in other environmental factors, such as pH and the concentration of dissolved oxygen may also be relevant, as unlike endoparasites, ectoparasites are exposed to external conditions, which may influence temporal parasite population changes alongside temperature.

Temperate regions where studies on seasonal changes in monogenean populations have been undertaken include the Czech Republic (Dávidová et al., 2005; Blažek et al., 2008), England (Chappell, 1969; Davies 1967), Finland (Valtonen et al., 1990), Poland (Prost, 1963; Wierzbicka, 1974), South Africa (Madanire-Moyo et al., 2011), Turkey (Ozer & Erdem, 1999; Aydogdu, 2006), and the U.S.A. (Crane & Mizelle, 1968; Rawson & Rogers, 1972). With the exception of South Africa, these are all in northern temperate continental areas or are close to such continents, and thus experience wide ranges in temperature through the year (Duckson, 1987). In contrast, New Zealand is an isolated set of islands and does not experience the same extremes in temperatures as in continental climates (Green et al., 1987). New Zealand is described as having a mild oceanic climate. That is, it is generally, wet, windy, and most notably experiences a relatively narrow range in annual temperatures, with mild summers and winters. As such, New Zealand lakes experience a narrower range of annual temperature changes relative to those at similar latitudes in the Northern Hemisphere (Green et al., 1987). The unique seasonal and climatic characteristics of New Zealand and its lakes makes it an ideal location in which to examine temperature effects on monogenean seasonality.

The western mosquitofish, *Gambusia affinis*, native to the south-eastern United States and Mexico, has established non-native populations in a number of locations globally (Pyke, 2008). *Gambusia* live in stagnant or slow moving waters with wide temperature ranges (Rivas, 1963) and can tolerate temperatures from 0-39 °C (Cherry et al., 1976), though they typically prefer water temperatures from 31-35 °C (Pyke, 2005), and will undertake diurnal migrations seeking areas of preferred temperature (Maglio & Rosen, 1969; Winkler, 1979). Examples of annual air temperature ranges in the native distribution of *G. affinis* between 1991 and 2020 include an average annual minimum of -4.4 °C to an average annual maximum of 31.7 °C in southern Illinois, U.S.A. (NOAA 2023). In Texas, U.S.A., the average annual minimum temperature from 1991 to 2020 was 3.6 °C and the average annual maximum was 34.2 °C (NOAA 2023). Such a tolerance to wide temperature ranges has allowed *G. affinis* to successfully establish populations across much of the world (Pyke, 2008). *Gambusia affinis* plays host to several monogenean species, including *Salsuginus seculus*, *Salsuginus bermudae* Rand & Wiles, 1987, and *Gyrodactylus gambusiae*. The monogeneans of *Gambusia affinis* have been the subject of various ecological studies (Mizelle & Arcadi, 1945; Rogers & Welborn, 1965; Hanek & Fernando, 1972; Rand & Wiles, 1987; Nitta & Nagasawa, 2014; McAllister et al., 2015;

Vasquez, 2016; Carpenter & Hermann, 2020), however, the seasonal changes of prevalence and intensity of specific species not previously studied.

Prevalence and intensity of monogenean infestations may change with season, but the likelihood and intensity of infestations may vary from host to host within a single species based on characteristics of the host individual. For example, it has been suggested that larger individual fish within a population may be infested more frequently and may harbour greater numbers of ectoparasites, though evidence for this is variable (Kuris et al., 1980; Rohde et al., 1995; Poulin, 2000; Rubio-Godoy, 2008; Madanire-Moyo et al., 2011). Large fish present a greater surface area onto which monogeneans may attach and therefore, offer a more favourable habitat patch. Larger fish will also typically be more mature, and as such have had more time to acquire parasites. In the case of *G. affinis,* individuals reach a maximum length of 60 mm (McDowall, 1990) and usually live no longer than 15 months (Pyke, 2008). As such, individuals that have reached a length of 50 mm or more are likely to be quite mature. On this basis, *G. affinis* individuals that are large and old would be expected to see greater parasite prevalence and intensity. However, patterns in host age and parasite infection are not universal and younger individuals may have more parasites than their older counterparts (Wunderlich et al., 2022). Testosterone is often considered to suppress immune function (Foo et al., 2016, Roved et al., 2017) and as male teleosts have higher levels of testosterone than females (Borg, 1994), it is possible that more males will be infested with monogeneans and have higher intensities of infestation. This has been observed in *Salmo trutta* L., where sexually mature males had higher prevalence and intensity of ectoparasites (Pickering & Christie, 1980). Little research appears to exist on parasite differences between sexes in fish. As *G. affinis* shows marked sexual dimorphism, with mature males possessing a distinct modified anal fin for insemination known as the gonopodium and being overall smaller than females (McDowall, 1990), it presents an ideal model species on which to test the hypothesis that males suffer from greater infestation by monogeneans. *Salsuginus seculus* and *Gyrodactylus gambusiae* have recently been reported infesting *Gambusia affinis* in New Zealand (Renner & Duggan, 2023).

The aims of this study were to investigate the temporal changes in monogenean populations on *G. affinis* in ponds on the Hamilton campus of the University of Waikato, recording their prevalence and mean intensity on a monthly basis. Further, to determine whether the size or sex of *G. affinis* hosts has any effect on the prevalence or intensity of *S. seculus*, these host characteristics were tested in relation to the aforementioned parasitological indices.

Materials and methods

From April 2022 to March 2023, sampling for *G. affinis* was carried out in Knighton and Oranga lakes (37°47'08.7"S, 175°18'53.3"E and 37°47'12.4"S, 175°18'57.2"E, respectively) two connected ponds on the Hamilton campus of the University of Waikato, New Zealand. Minnow traps were set at five sites around the shoreline of the ponds on a monthly basis; two in Oranga Lake, one in the connecting stream, and two in Knighton Lake (Figure 1). In April and May 2022, traps were left at each site for two to four hours. However, due to decreasing numbers of fish caught throughout the remainder of the study, subsequent traps were left for approximately 24-hours. Counts of *G. affinis* from the traps on a monthly basis gave a catch per-unit effort (CPUE), expressed as numbers caught per hour, to infer the seasonal dynamics of the fish. It was aimed that at least 50 individuals from each of the two lakes and the stream would be caught (i.e., a total of 150 per month). In certain cases, it was necessary to supplement fish capture from the traps with the use of a pole mounted net. This was necessary when fish numbers were low, or when the traps had failed to capture adequate numbers of fish, something that notably occurred during, or immediately following rain. On a few occasions (Knighton Lake in May, June, September, October, November, and December 2022, and January 2023; Oranga Lake in June and August 2022, and the stream in June and October 2022), fish were scarce, and thus less than 50 fish were examined for monogeneans from those sites on those dates. During winter, in July 2022 for Oranga Lake and Knighton Lake and in August 2022 for the connecting stream, no fish could be collected. The lowest number of fish collected was 50, from the stream in July 2022. Environmental factors were measured when fish were collected. At the water's surface, pH was measured using a pHTestr handheld pH meter. Temperature (°C) and dissolved oxygen concentration ($mg.L^{-1}$) were measured 15 cm below the surface at each of the five sites using YSI ProSolo, YSI Pro 2030, and YSI 55 meters. For chlorophyll *a* analysis, 20 mL water samples were drawn from below the water's surface at each of the five sites and passed through a glass microfiber filter (nominal pore size 0.5 μm). Each filter was folded, wrapped in aluminium foil, and frozen at -20 °C until analysed. Extraction and analysis of chlorophyll *a* were carried out in low light to avoid chlorophyll degradation. Each filter was blended in 20 mL of 90% $MgCO_3$-buffered acetone using an Ozito homogeniser, and the resulting slurry was placed in a centrifuge tube. Samples were refrigerated at 4° C and left to settle for anywhere between 2-24 hours. After this, the samples were shaken and centrifuged in a Universal 320 R centrifuge at 1600 *g* at high-brake for 10 minutes. A Turner Designs 10-AU fluorometer was used to measure chlorophyll *a* concentration. Fluorescence of 5 mL aliquots of solution were measured in the fluorometer. In some instances of especially high chlorophyll *a* concentration it was necessary to dilute the solution by half. To compensate for interference from chlorophyll *a* degradation products, 150 μL of 0.1 N HCl was added to samples and the fluorescence measured again. Once fish were captured and counted, they were transported to the laboratory and anaesthetised with an overdose of benzocaine. Once the fish were deceased, they were measured for body length, weighed, their sex determined based on the presence or absence of the gonopodium, and their outer surface was inspected for parasites using an Olympus SZ40 dissecting microscope. Gills were then removed and examined under the microscope for parasites. Identification of parasites is described in Renner and Duggan (2023).

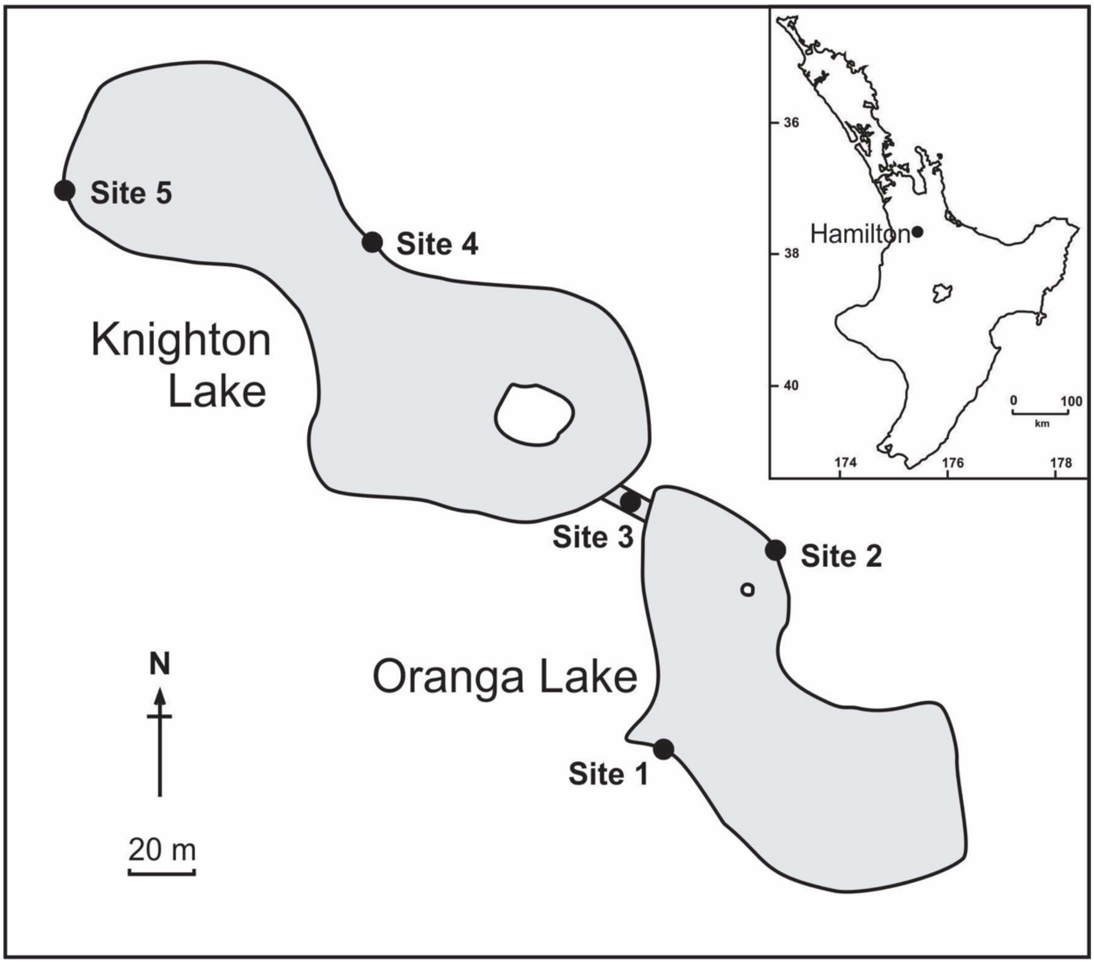

Figure 1. Locations of the five sampling sites in Knighton and Oranga Lake at the University of Waikato, Hamilton campus.

Statistical analyses

Prevalence and mean intensity of *S. seculus* were analysed according to Bush et al. (1997) where the prevalence is the number of host individuals infested by a given parasite species and the mean intensity is the average number of parasites of a single species occurring on infested hosts. To determine if there was a significant difference in *S. seculus* prevalence between months, a χ^2 test was used. Spearman correlation analysis was used to examine the association between environmental variables, fish numbers, and the prevalence and mean intensity of *S. seculus* across months. Analysis of covariance (ANCOVA) on a Poisson general linear model was used to compare the size (length and weight) and sex of the fish in the presence and absence of *S. seculus*. All analyses were performed in R version 4.3.1 (R Core Development Team 2023).

Results

Across the course of the year, 1293 *G. affinis* were examined for monogenean parasites, and from these 551 *S. seculus* individuals and 14 *Gyrodactylus gambusiae* individuals were encountered. The lowest average water temperature of 12.3 °C occurred in June (Figure 2). The maximum recorded water temperature was 23.7 °C in January. The pH level was lowest in June and September at 7.2 and was highest in November with a level of 8.6 (Figure 3). The concentration of dissolved oxygen changed regularly, increasing and decreasing from month to month, with no discernible pattern (Figure 4). The lowest concentration of chlorophyll *a* occurred in May of 5.6 $\mu g\ L^{-1}$, while the highest concentration, 118.5 $\mu g\ L^{-1}$, was recorded in December (Figure 5). Fish numbers (CPUE) caught in traps were highest in autumn but dropped off throughout the rest of the year, with no fish caught in traps in November (Figure 6). The highest prevalence of *G. gambusiae* occurred in March, at 2.9% and the intensity was 1 in all but one instance in March, when two *G. gambusiae* individuals were observed on a fish. The prevalence of *S. seculus* changed significantly over the course of the year ($\chi^2 = 141.74$, df = 11, $P<0.001$), being highest in December 2022 when 41.0% of fish were infested with *S. seculus* and lowest in September at 4% (Figure 7). The mean intensity did not exhibit a clear pattern and was high in both late autumn and early to mid-summer (Figure 8). The mean intensity was highest in July 2022, with an average of 3 *S. seculus* individuals per infested host. Mean intensity was at its second highest level in December 2022, with an average of 2.6 *S. seculus* individuals per infested host. The highest intensity for a given *Gambusia affinis* individual was in January, when one fish was found to host 12 *S. seculus* individuals. Of the environmental variables measured, the concentration of chlorophyll *a* ($S = 36.56$, $\rho = 0.87$, $P < 0.001$) primarily, and secondarily the pH ($S = 104.41$, $\rho = 0.63$, $P = 0.020$), showed the greatest degree of correlation to the prevalence. No other environmental variable was significantly correlated to prevalence, including temperature, and the fish numbers. Neither the fish numbers, nor any measured environmental variable showed a significant correlation to mean intensity. From the campus ponds, 503 male and 790 female *G. affinis* were examined for parasites over the course of the year. The average length of male fish was 2.5 cm and the average weight was 0.1189 g. The average length of female fish was 2.9 cm and the average weight was 0.2346 g. A total of 61 *S. seculus* individuals were found from males and 473 were recorded from females. The prevalence was significantly different between the sexes ($\chi^2 = 133.72$, df =1 $P<0.001$). The results of the ANCOVA indicated length of the fish was the greatest predictor of parasite numbers found on fish (Std. error = 0.0015, z value = 4.13, P<0.001), with heavier fish also having more parasites (Std. error = 1.37, z value = 1.98, P = 0.047). Differences in parasite statistics by sex are summarised in Table 1.

Table 1. Prevalence and mean intensity of *S. seculus* for female and male *G. affinis* across all months from the Hamilton campus of the University of Waikato.

	Male	Female
Prevalence	9.34%	27.31%
Mean intensity	1.30	2.19

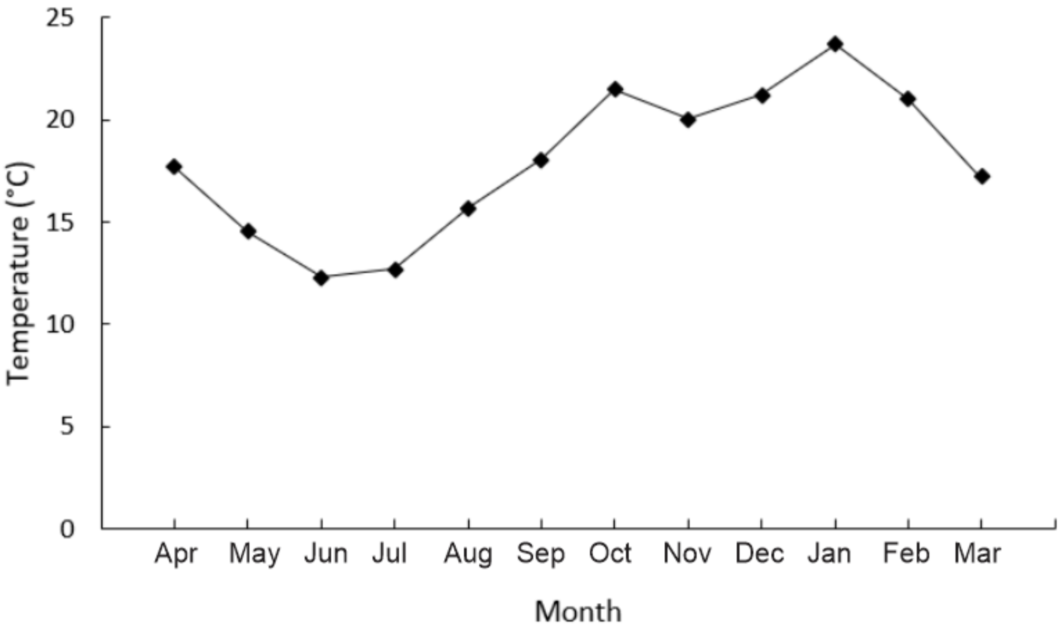

Figure 2. Average water temperature (°C) across the five sampling sites at a depth of 15 cm from the University of Waikato, Hamilton campus lakes from 2022-2023.

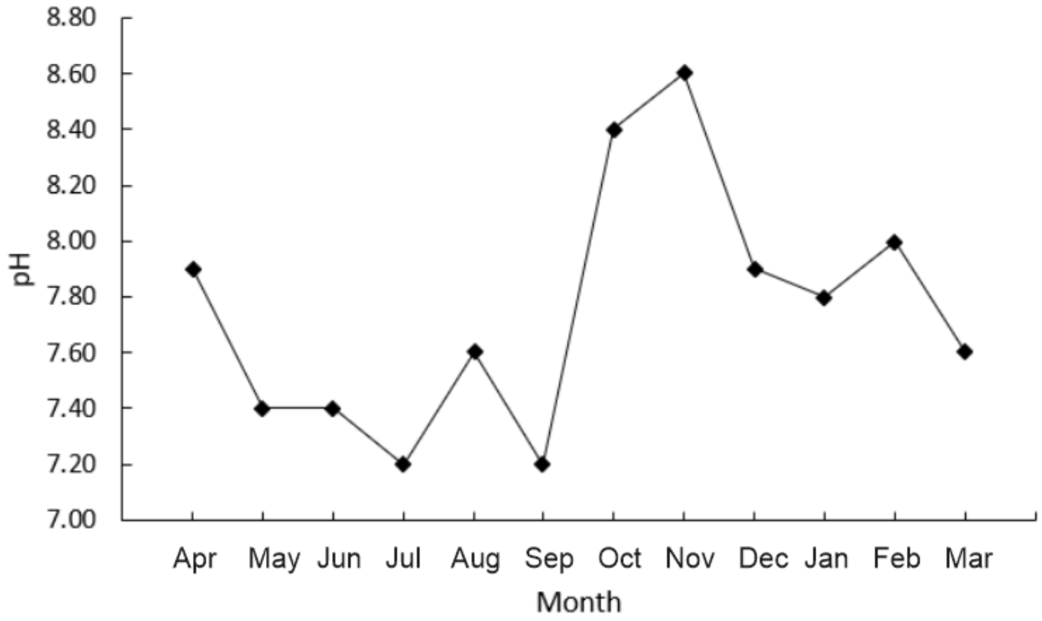

Figure 3. The average pH across the five sampling sites at the water's surface from the University of Waikato, Hamilton campus lakes from 2022-2023.

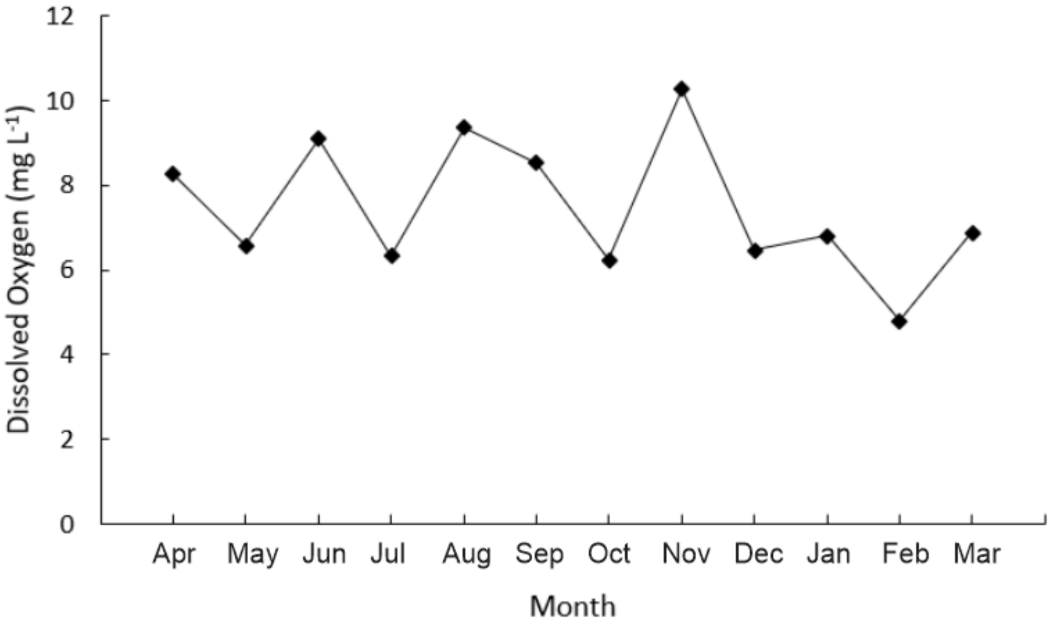

Figure 4. Average dissolved oxygen (mg L^{-1}) across the five sampling sites 15 cm below the water's surface from the University of Waikato, Hamilton campus lakes from 2022-2023.

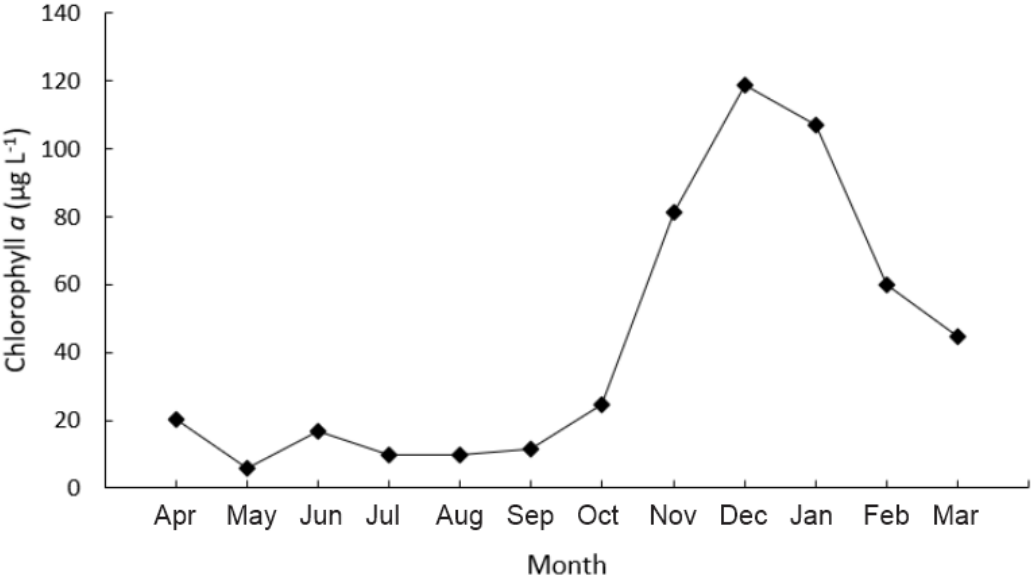

Figure 5. Average chlorophyll *a* concentrations (µg L^{-1}) across the five sampling sites from the University of Waikato, Hamilton campus lakes from 2022-2023.

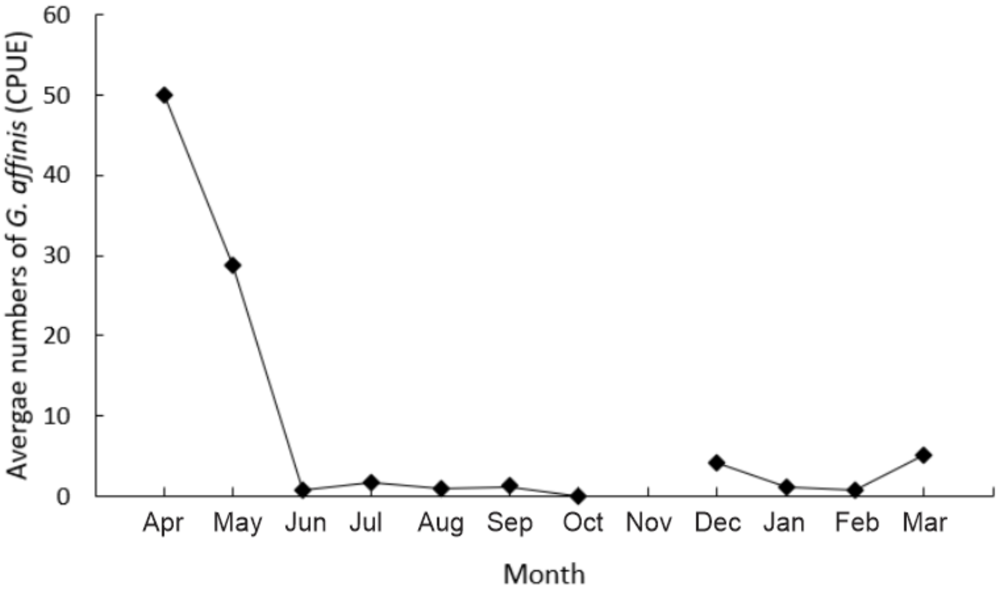

Figure 6. Average *G. affinis* numbers catch per unit effort (numbers of fish per trap per hour) from the University of Waikato, Hamilton campus lakes from 2022-2023. The missing value in November was due to no fish being caught in traps on this month.

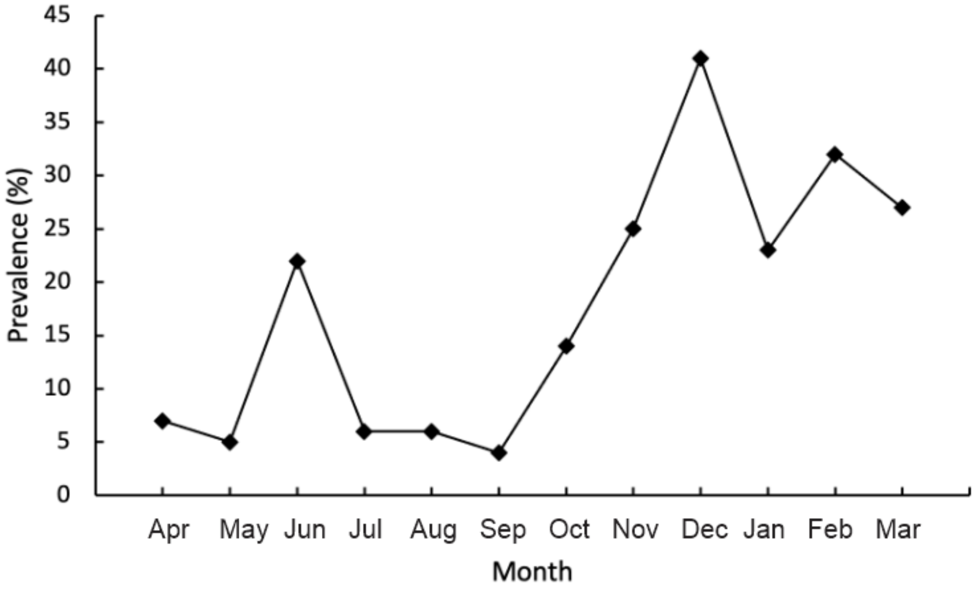

Figure 7. Prevalence for infestation of *G. affinis* by *S. seculus* from 2022-2023 in the University of Waikato, Hamilton campus lakes.

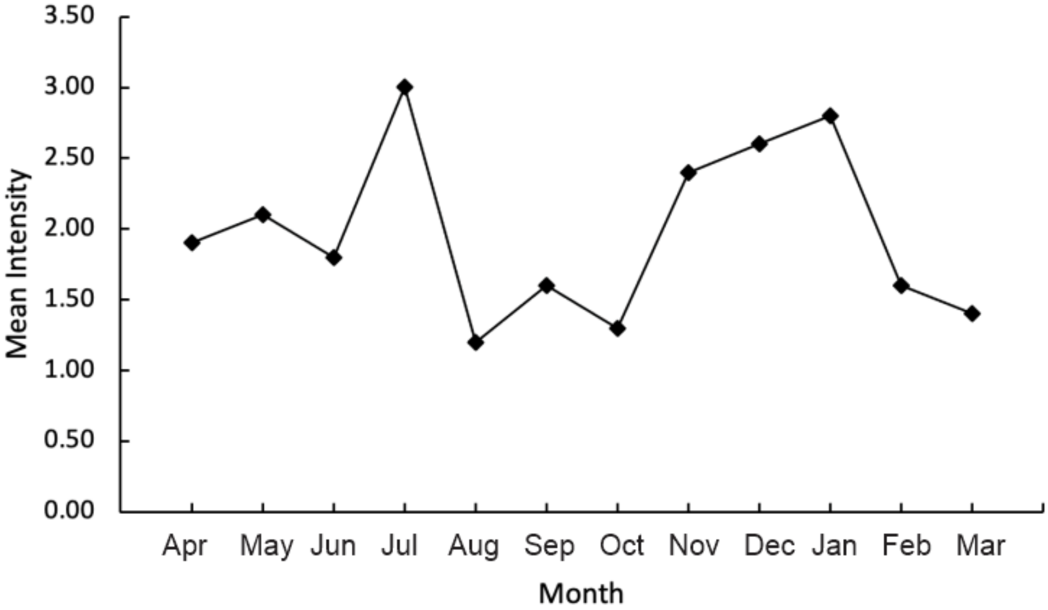

Figure 8. Mean intensity for infestation of *G. affinis* by *S. seculus* from 2022-2023 in the University of Waikato, Hamilton campus lakes.

Discussion

Over the year, the change in prevalence of *S. seculus* showed a noticeable and significant seasonal pattern. The changes in mean intensity were more erratic and did not change to as great an extent. This indicates that, with occasional exceptions, infested fish generally had 1-3 *S. seculus* individuals on their gills throughout the year. The highest intensity was a *G. affinis* individual caught in January which was infested with 12 *S. seculus* individuals. Though the intensity might have been largely consistent among fish, the prevalence was not. Numbers of infested individuals showed a major increase over the spring reaching their peak in the summer. This summer increase in the monogenean population reflects what has been seen in studies from continental climates (Ozer & Erdem, 1999; Aydogdu, 2006; Poulin, 2020), despite the narrower temperature range in New Zealand. The fact that this would then correlate with chlorophyll *a* concentration in the campus ponds is therefore, not especially surprising. Pond productivity would have increased heading into the summer. The correlation of prevalence with pH is probably for the same reason as algal blooms and periods of rapid photosynthesis can raise the pH of the water (Wetzel, 2001). Other environmental variables did not show significant correlation with the parasitological indices over the course of the year. Despite temperature being implicated in the changes in monogenean populations in other studies (Valtonen et al., 1990; Ozer & Erdem, 1999; Davidova et al., 2005; Aydogdu, 2006; Blažek et al., 2008), it was not correlated to the seasonal changes in *S. seculus* prevalence. This is in agreement with Poulin (2020) who found that temperature was not actually the driving factor in seasonal monogenean population changes in temperate climates. It is unlikely that temperature has no effect at all, as it has been shown quite clearly to be relevant to the reproduction and development of oviparous monogeneans (Gelnar, 1987; Zhang et al., 2022). However, in New Zealand with its mild climate and the narrow temperature range of 12 °C observed here, biotic factors may take precedence over abiotic ones. It seems more likely that the changes in population size and behaviour of *G. affinis* affect the prevalence and intensity of *S. seculus*. However, correlation tests did not show a correlation between the fish numbers caught in minnow traps each month and the prevalence and mean intensity of *S. seculus*. Nevertheless, it is possible that this was due to the difficulty in collecting fish, particularly following rain, when fish capture became more difficult. What correlations do exist for environmental variables show that prevalence increased with chlorophyll *a* and to a lesser extent, pH. It is reasonable to propose that this is a coincidental correlation and is merely occurring because the fish numbers have become greater with the breeding season of *G. affinis*, which extends from mid-Spring to mid-Autumn (Pyke, 2005). As chlorophyll *a* represents a measure of algal concentration, this would indicate more food for grazing zooplankton, which in turn provides food for *G. affinis* (Pyke, 2005). *Gambusia affinis* did become more visible in large shoals over the summer months through until mid-autumn, evidently due to their annual population increase. With the assistance of hand netting, a number of *G. affinis* were caught over these months even when the traps failed, as at this time many were gathering under vegetation, possibly a diurnal change or in association with breeding (Maglio & Rosen, 1969; Winkler, 1979; Pyke, 2005). With the nature of *G. affinis* breeding in mind, the patterns of prevalence appear to largely coincide with this factor. A rapid increase in the number of host individuals in the environment would naturally support an increase in their parasites and this is what is predicted by parasite ecology (Bagge et al., 2004). Additionally, the act of breeding and associated courting behaviour would promote the transmission of parasites. Breeding *G. affinis* individuals gather closely together and make physical contact, such as when males contest with one another and in the act of copulation itself (Pyke, 2005). These behavioural changes and the regular seasonal increases and decreases of *G. affinis* populations are therefore, the most probable primary cause for the population changes in *S. seculus*.

Female *G. affinis* had the greatest prevalence and mean intensity of *S. seculus*, which did not support the hypothesis of lower male immunocompetence in this species as observed in *Salmo trutta* (Pickering & Christie, 1980). Instead, fish length and weight was the major predictor of prevalence and intensity in *G. affinis*. The fact that females were the hosts of the majority of *S. seculus* individuals found is likely related to the size discrepancy between male and female *G. affinis* as the female fish are considerably larger (Mcdowall, 1990; Pyke, 2005). At first glance, the size to parasite relationship in *G. affinis* seems a simple one, as larger fish present larger and higher quality habitat patches (Kuris et al., 1980), and bigger targets for swimming oncomiracidia, although the relevance of fish size to parasite abundance is difficult to determine (Poulin, 2000; Poulin, 2013). However, there is more to be considered in this situation; *G. affinis* have a short life cycle, typically reaching an age of 12-15 months, and a maximum of age of 18 months in rare cases (Pyke, 2008). Fish that have reached 50 mm in length likely represent particularly mature individuals (McDowall, 1990), and therefore, should be more immunologically competent (Izhar et al., 2015). In fact, for this reason, younger fish are expected to be parasitised more than older fish (Ashby & Bruns, 2018; Wunderlich et al., 2022). It is thus difficult to be sure why the larger and older fish had the greatest prevalence and intensity of *S. seculus*. Perhaps this is an effect of focusing on a single parasite species and should the view be broadened to the parasite communities of *G. affinis*, one would find younger fish to host more parasites overall. In the case of short lived fish like *G. affinis*, this may be a result of the time required to acquire parasites. Though the young fish may be more vulnerable to infestation by monogeneans, *G. affinis* grow rapidly (Pyke, 2005) and therefore, may already be of a considerable size before monogeneans have had an opportunity to attach themselves. As weight was found to be a significant predictor of prevalence and intensity for individuals throughout the year, it is worth noting that without examining the fish for all parasite taxa, it cannot be known for certain what portion of the mass is contributed by the fish and what portion is contributed by internal parasites (Timi & Poulin, 2020). A heavier fish may already experience morbidity from an unseen parasite load that could predispose it to infestation or infection by further parasites. It is possible that such a situation contributed to the greater monogenean infestation of heavier *G. affinis* but the extent of such a contribution would require further research.

Of the two monogenean species observed in this study, *S. seculus* was by far the most abundant. Why *Gyrodactylus gambusiae* had such a low abundance is difficult to ascertain. There does not seem to be any reason to expect greater monogenean numbers on the gills than the skin (Scheifler et al., 2022). This species has remained little studied since its initial description. However, Carpenter and Hermann (2020) examining parasite communities of *Gambusia affinis* in Texas, observed *S. seculus* and an unidentified *Gyrodactlyus*. This was almost certainly *G. gambusiae* as this is the only *Gyrodactylus* species known from *Gambusia affinis* (Hoffman, 1999). In this instance also, the abundance of *Gyrodactylus* was considerably lower than *S. seculus*. It is difficult to be sure from these two studies why this discrepancy in abundance might be. However, differences in transmission may be responsible. *Salsuginus seculus*, like most monogenean species, is oviparous (Whittington & Chisholm, 2008) and releases eggs directly into the water column, which hatch into free-swimming larvae that then find their host. *Gyrodactlyus*, in contrast, is a viviparous genus that lacks a specific transmission stage. Instead, parents give birth to crawling, sexually mature young, that attach onto the same host as their parent (Bakke et al., 2007). Transmission to another host may take place at any point in the parasite's life. Continuous transmission throughout the parasite's life enhances the utilisation of host resources; however, transfer from one host to another is usually not undertaken by parasites as it is a risky manoeuvre and can result in high mortality (Bakke et al., 2007; Tepox-

Vivar et al., 2022). Transmission may be achieved in a number of ways. If a host and prospective host make physical contact then monogeneans may cross from one host to the other (Bakke et al., 1992; Bakke et al., 2007). These monogeneans may also detach and drift into the water column where they may remain as long as possible waiting for a potential host, or they may attach themselves to a substrate to wait for a prospective host (Bakke et al., 1992; Bakke et al., 2007). There are then, two monogenean species here with distinctly different abilities to transmit from host to host. The free-swimming larval stage of *S. seculus* may have lower mortality in its transmission stage than *Gyrodactylus gambusiae*, but *G. gambusiae* has many more strategies apparently open to it, and on this basis would be expected to become the more abundant monogenean in this freshwater system. In other instances, *Gyrodactylus* has been observed to spread extensively through host populations, and their prevalence is often high (Cone & Roth, 1993; Appleby, 1996; Mo, 1997; Dávidová et al., 2005). The fact that *G. gambusiae* had a lower prevalence than *S. seculus* may be due to a lack of attempts at transmission. Instead, *G. gambusiae* individuals may see higher aggregation and proliferate on single hosts leading to heavily burdened individuals with higher mortality. One heavily burdened individual of *Gambusia affinis* was observed in April (Duggan, University of Waikato, personal observation). Why *Gyrodactylus gambusiae* would apparently have such a low abundance when other members of the genus do not, requires further investigation.

To conclude, a number of factors were considered that might affect the prevalence and abundance of monogeneans on *Gambusia affinis* in relation to seasonal changes and also host characteristics. Abiotic conditions did not appear to have a great effect on the populations of *S. seculus*, but seasonal variations were still seen as in locations where temperature was observed to vary to a greater degree. Instead, it seems that the seasonal population density increase in *G. affinis* and their close association when breeding may have led to increases in the numbers of *S. seculus*. The size of *G. affinis* was a predictor of prevalence and intensity of *S. seculus*. The possible reasons for this are diverse but the most simple explanation is that larger host individuals represent better habitat patches for ectoparasites, with larger animals having larger gills. The time available for parasites to accumulate is also greater for older fish, which have been alive longer, and hidden parasites might contribute.

This research has extended the knowledge of monogeneans of *G. affinis* and represents the first study on the ecology of *S. seculus* and *Gyrodactylus gambusiae*. It also extends the knowledge of monogeneans in New Zealand and has utilised this environment to examine parasite seasonality in a different climate to where most previous studies have been conducted.

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Chapter Three

A Survey of Monogenean Parasites Infesting Non-Native Freshwater Fish Species in New Zealand

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Abstract

Twenty-one non-native freshwater fish species have established populations in New Zealand, yet only two of these species have been reported with monogenean infestations. The first of these was the grass carp *Ctenopharyngodon idella* (Valenciennes in Cuvier & Valenciennes, 1844) on which two monogeneans were reported. Recently, two monogenean species have been reported infesting the non-native mosquitofish, *Gambusia affinis* (Baird and Girard, 1853) in New Zealand. Based on the diversity of monogenean species known from New Zealand's non-native freshwater fish species in their native and other invaded ranges, it is likely that many monogeneans could be extant in the country, which remain unreported. On this basis, a survey of monogeneans from seven freshwater fish species, equivalent to one-third of New Zealand's non-native freshwater fish community, was undertaken. Eleven monogenean species were reported infesting non-native freshwater fish. Of these, nine were previously unreported from New Zealand, with one representing an entirely new genus to the country. It has been hypothesised that younger fish should show a higher susceptibility to parasitism and disease; thus, in the course of this survey two age classes of goldfish, *Carassius auratus* (L.), were compared on the basis of the prevalence and intensity of their *Dactylogyrus* infestations. Juvenile *C. auratus* had significantly higher levels of prevalence and intensity than their adult counterparts, supporting the hypothesis that juvenile fish are more susceptible to parasite infestation.

Introduction

Monogeneans are common parasites of freshwater fishes (Whittington & Chisholm, 2008). This class of platyhelminths attach themselves to the surface of their hosts using a specialised organ on their posterior end, known as the haptor (Whittington & Chisholm, 2008). Most monogeneans species are ectoparasites of fish, found on the gills or other outer surfaces (Whittington & Chisholm, 2008), though some species represent fish endoparasites (Llewellyn, 1960) or ectoparasites on other aquatic animals (Stunkard, 1924). One of the distinguishing characteristics of monogeneans is their direct single host life cycle; an individual monogenean will attach itself to a single host in its lifetime, utilising no intermediate hosts (Whittington & Chisholm, 2008). Additionally, any one monogenean species typically has only a single host species; that is, different fish species are unlikely to host the same monogenean species (Poulin, 1992; Whittington & Chisholm, 2008).

The freshwaters of New Zealand have seen the establishment of 21 non-native fish species as a result of introductions by humans (McDowall, 1990; Duggan & Collier, 2018). Non-native fish species have been introduced for a variety of reasons such as for sport and for ornamental purposes (Duggan & Collier, 2018). Some species are considered to be harmful in New Zealand's waters, including *Cyrpinus rubrofuscus* Lacépède, 1803 (koi carp), *Carassius auratus* (goldfish) and *Gambusia affinis* (mosquitofish). Establishment of these species has been linked to reductions in water quality through food web interactions and resuspension of sediment (Rowe, 2007).

Until recently, only three monogenean species had been identified from New Zealand freshwater fishes, of which just two were identified to the species level (Hine et al., 2000). Two monogenean species were identified from the non-native *Ctenopharyngodon Idella* in the 1970s. These were *Dactylogyrus ctenopharyngodonis* from the gills and *Gyrodactylus ctenopharyngodontis* from the scales and fins. These were recorded from a consignment of *C. idella* from Hong Kong, and treatment with quinine was reported to eliminate the monogeneans (Edwards & Hine, 1974). However, lack of subsequent research makes it unclear if these monogeneans are still extant in New Zealand. An undescribed *Gyrodactylus* species from the gills of the native *Galaxias brevipinnis* Günther, 1866, is also known from New Zealand (Hewitt & Hine, 1972). Most recently, Renner and Duggan (2023) reported two further monogenean species from New Zealand, infesting non-native *Gambusia affinis*; *Salsuginus seculus* from the gills, and *Gyrodactylus gambusiae*, from the gills and fins. Renner and Duggan (2023) suggested that many additional monogenean species have had the potential to establish populations in New Zealand, co-introduced with their non-native fish hosts now established in New Zealand freshwaters, but that surveys for this group have been limited. As monogeneans are highly host specific, each fish species may possess unique monogenean taxa not known from New Zealand.

The aims of this study were to survey a range of non-native freshwater fish species in New Zealand for monogenean parasites. Additionally, because parasite infection and infestation are known to vary with host size (Poulin, 2013) and with host age (Ashby & Bruns, 2018; Wunderlich et al., 2022), different age classes of *C. auratus* collected were used to investigate the difference in parasite prevalence and intensity in relation to these host factors.

Materials and methods:

Seven non-native freshwater fish species were selected on the basis of being the most common and abundant in freshwaters of the Waikato region and partly out of opportunism; *Carassius auratus* (goldfish), *Cyprinus rubrofuscus* (koi carp), *Gambusia affinis* (mosquitofish), *Ameiurus nebulosus* (Lesueur, 1819) (brown bullhead catfish), *Scardinius erythrophthalmus* (L.) (rudd), *Perca fluviatilis* (L.) (European perch), and *Tinca tinca* (L.) (tench). The studied fish species varied in year of introduction and native range, as well as the dates on which they were collected (Table 1). Juvenile *C. auratus* (2.4-3.8 cm long) were collected using minnow traps on 18 November 2022 and 5, 6, and 7 January 2023 from Oranga Lake and Knighton Lake on the Hamilton campus of the University of Waikato (37°47'12.4"S, 175°18'57.2"E and 37°47'08.7"S, 175°18'53.3"E, respectively), and from Lake Ngaroto (37°57'15.0"S 175°17'19.8"E) on 6 December, 2022. Monogenean species had previously been identified from *Gambusia affinis* by Renner and Duggan (2023) but this was from a single site. To determine if these and other monogenean species would be found elsewhere in the invaded range of *G. affinis*, fish were collected from Lake Ngaroto on 6 December 2022, using minnow traps left overnight, and Lake Puketirini (37°34'02.6"S 175°08'22.4"E) on 12 December, 2022, with a pole mounted net. A single juvenile *A. nebulosus* was caught from Knighton Lake on 6 January, 2023 in a minnow trap. Adult *A. nebulosus* were captured using fyke nets left in Knighton Lake for 24 hours on 3 March, 2023. They were also collected using three fyke nets set from the shore of Lake Waahi (37°34'12.6"S 175°07'57.8"E) left for 24 hours on 9 March and 11 May, 2023. *Perca fluviatilis*, *S. erythrophthalamus*, and sub-adult *Cyprinus rubrofuscus* were also collected in these fyke nets on these dates from Lake Waahi. Fish caught from Lake Waahi in May were kept alive in an approximately 7 m^3 tank at the University of Waikato Aquatic Research Centre, until such time as they were removed and humanely euthanised with an overdose of benzocaine. All other fish collected prior to this were euthanised with an overdose of benzocaine and examined on the day they were caught. *Cyprinus rubrofuscus* caught via electrofishing from Lake Waikare (37°26'00.4"S 175°12'00.1"E) on 6 March were also examined and, in this case, specimens had been frozen prior to examination. *Carassius auratus* adults (21.0-25.6 cm long) and *Cyprinus rubrofuscus* that were being held in the tank for unrelated work at the University of Waikato Aquatic Research Centre, were also examined in this research. These fish had been sourced from various locations including Lake Ngaroto and the Waikato River and individuals had been held there for up to five years. One *P. fluviatilis* and one *T. tinca* were caught from Lake Tomarata (36°11'36.5"S 174°39'00.7"E) in April and added to this tank during the course of the research. Fork length measurements were made of *C. auratus* individuals. In the examination of monogenean parasites, euthanised fish had their gills removed using a pair of forceps which were immersed in water and observed under an Olympus SZ40 dissecting microscope. Photography of parasites was performed with a DMRE Leica microscope.

Identification of monogenean species was achieved by comparison of morphology with previous descriptions from the literature (Klassen & Beverly-Burton, 1985; Pugachev et al., 2009; Nitta & Nagasawa, 2015; Tu et al., 2015; Ling et al., 2016; Cloutman et al., 2018; Trujillo-Gonźalez et al., 2018; León et al., 2019; Tancredo & Martins, 2019; Vancheva et al., 2020). Measurements of sclerotised structures in *Dactylogyrus* species were made for the total length of the hamulus, length to hamulus notch, blade length, inner root length, outer root length, gap length, bar width, bar length, penis length, and accessory piece length. The body length and width were also measured. The gap ratio was also calculated as the ratio of the gap length to the blade length. These measurements were made following the methods of Trujillo-Gonźalez et al. (2018). Measurements of the male copulatory complex could not always be made as it was not visible in all species or individuals. Measurements of sclerotised structures of a *Ligictaluridus* species were made for the total hamulus length, the length to notch, blade length, length of superficial root, ventral bar width and length, dorsal bar length and width, penis length and diameter, and accessory piece length. These measurements are given in micrometres and were made according to Nitta and Nagasawa (2015).

Table 1. Native ranges, year of introduction to New Zealand, and date of collection for fish examined in this study (McDowall, 1990; Duggan & Collier, 2018).

Fish	Native range	Year(s) of introduction to New Zealand	Date of collection
Carassius auratus (goldfish)	Eastern Asia	1864, 1867, possible subsequent introductions	18 Nov 2022 (campus lakes); 6 Dec 2022 (Lake Ngaroto); 5, 6, 7 Jan 2023 (campus lakes)
Ameiurus nebulosus (brown bullhead catfish)	Southern Canada, northern United States east of the Rocky Mountains, Florida, Texas	1877	6 Jan 2023, 3 March 2023 (campus lakes); 9 March 2023; 11 May 2023 (Lake Waahi)
Cyprinus rubrofuscus (koi carp)	Central Asia	c. 1960	6 March 2023 (Lake Waikare), 9 March 2023; 11 May 2023 (Lake Waahi)
Scardinius erythrophthalmus (rudd)	United Kingdom, Scandinavia, western Europe, Russia, central Asia	c. 1967	9 March 2023; 11 May 2023 (Lake Waahi)
Perca fluviatilis (perch)	Europe	1868, 1870, 1877	9 March 2023 (Lake Waahi); April 2023 (Lake Tomarata); 11 May 2023 (Lake Waahi)
Gambusia affinis (western mosquitofish)	Northern Mexico and south eastern USA	1867, 1868	6 Dec 2022 (Lake Ngaroto), 12 Dec 2022 (Lake Puketirini)
Tinca tinca (tench)	Europe	1867, 1868	April 2023 (Lake Tomarata)

Statistical analyses

Bush et al. (1997) defines prevalence as the proportional number of host individuals infested by a single parasite species and the mean intensity as the average number of parasites of a single parasite species occurring on an infested host. For monogeneans of *Carassius auratus*, it was not possible to identify all the individual parasites to species level due to the fact they were found in high numbers and comprised a variety of *Dactylogyrus* species. As such, the parasitological indices described above have been applied at the genus level. This is considered justified based on Poulin (2018) as the species were all from the genus *Dactylogyrus*, all were attached to the gills, and all had the same feeding strategy (Whittington & Chisholm 2008). Differences in prevalence between the two age classes were statistically analysed using a χ^2 test, and differences in the mean intensity were analysed by using a bootstrap Welch two-sample t-test. All analyses were performed in R version 4.3.1 (R Core Development Team 2023).

Results

Overall, eleven monogenean taxa were observed, including nine previously unreported in New Zealand. Eight of the nine previously unreported taxa could be identified to the species level. From *A. nebulosus*, the ancyrocephalid, *Ligictaluridus pricei* (Mueller, 1936) (Figure 1) was identified infesting the gills. Six dactylogyrid monogenean species from *Carassius auratus* were identified. These were, *Dactylogyrus anchoratus* (Dujardin 1845) (Figure 2), *D. dulkeiti* Bychowsky, 1936 (Figure 3), *D. formosus* Kulweic, 1927 (Figure 4), *D. inexpectatus* Izyumova, 1955 (Figure 5), *D. intermedius* Wegener, 1909 (Figure 6), and *D. vastator* Nybelin, 1924 (Figure 7). A further *Gyrodactylus* species was also observed from the gills of *Carassius auratus*, that could not be confidently identified to species level (Figure 8). *Dactylogyrus inexpectatus* appeared to be the most common monogenean of *Carassius auratus. Dactylogyrus minutus* Kulweic, 1927 (Figure 9), was identified from *Cyprinus rubrofuscus*.

Measurements for monogeneans are given in Table 2 as the mean ± the standard deviation, with the range and number, *n*, given. All measurements are given in micrometres.

Measurements for features of *L. pricei* not found in *Dactylogyrus* and, therefore, not included in Table 2 are outlined as follows. Length of superficial root, 8.7 ± 2.1 (5–12; *n* = 14). Ventral bar, 37.9 ± 3.6 (33–44; *n* = 7) long, 4.9 ± 2.0 (3–8; *n* = 7) wide. Dorsal bar, 37.1 ± 2.8 (30–47; *n* = 7) long and 9.7 ± 1.7 (7–12; *n* = 7) wide. Diameter of penis, 2 (*n* = 1).

Table 2. Mean values standard deviations, ranges of measurements taken for seven *Dactylogyrus* and one *Ligictaluridus* species, as well as the gap ratio, recorded from non-native fish species in New Zealand (all measurements given in μm and number of measurements is given as *n*)

Species	*D. anchoratus*	*D. dulkeiti*	*D. formosus*	*D. inexpectatus*	*D. intermedius*	*D. minutus*	*D. vastator*	*L. pricei*
Body length	520 (*n*=1)	156±19.8 (142-170; *n*=2)	235±21.2 (220-250; *n*=2))	633±234.6 (280-1000; *n*=9)	222 (*n*=1)	335±102.4 (150-460; *n*=8)	679±8.0 (508-850; *n*=2)	514±144.8 (370-680; *n*=5)
Body width	88 (*n*=1)	63±10.6 (55-70; *n*=2)	55±7.1 (50-60; *n*=2)	218±97.5 (90-380; *n*=9)	86 (*n*=1)	49±11.0 (36-72; *n*=8)	195±5.0 (160-230; *n*=2)	108±38.6 (40-136; *n*=5)
Hamulus total length	110±1.4 (109-111; *n*=2)	53±2 (51-55; *n*=4)	58±2.4 (55-60; *n*=4)	39±7.5 (23-49; *n*=13)	25±0 (*n*=2)	44±2.5 (41-48; *n*=14)	33±2.4 (31-37; *n*=6)	41±3.9 (36-50; *n*=14)
Length to notch	72±0.7 (71-72; *n*=2)	30±0.5 (30-31; *n*=4)	36±1.7 (34-38; *n*=4)	29±2.3 (26-34; *n*=13)	20±0 (*n*=2)	39±1.9 (36-41; *n*=14)	32±2.0 (30-35; *n*=6)	38±3.7 (34-46; *n*=14)
Blade length	31±1.4 (30-32; *n*=2)	21±0.5 (20-21; *n*=4)	18±1.7 (15-19; *n*=4)	17±3.4 (12-25; *n*=13)	8±0 (*n*=2)	12±2.3 (8-17; *n*=14)	14±0.6 (30-35; *n*=6)	14±3.9 (6-18; *n*=14)
Inner root length	52±0 (50; *n*=2)	28±1.4 (26-29; *n*=4)	33±2.2 (30-35; *n*=4)	17±6.5 (10-36; *n*=13)	14±0 (*n*=2)	14±2.4 (9-17; *n*=14)	18±1.9 (15-20; *n*=6)	

Outer root length	3±0.7 (2-3; *n*=2)	2±0 (2; *n*=4)	2±0 (2; *n*=4)	4±0.8 (3-5; *n*=13)	4±0.7 (3-4; *n*=2)	5±1.1 (3-6; *n*=14)	11±0.8 (10-12; *n*=6)	
Gap length	96±2.8 (94-98; *n*=2)	40±1.0 (39-41; *n*=4)	47±2.2 (45-50; *n*=4)	31±6.3 (19-39; *n*=13)	22±1.4 (21-23; *n*=2)	38±2.3 (35-42; *n*=14)	37±1.4 (35-38; *n*=6)	
Gap ratio	6±0.3 (2.9-3.3; *n*=2)	2±0.1 (1.9-2; *n*=4)	3±0.2 (2.6±3; *n*=4)	2±0.5 (1-2.6; *n*=13)	3±0.1 (2.6-2.8; *n*=2)	3±0.7 (2-4.9; *n*=14)	3±0.1 (2.5-3; *n*=6)	
Bar width	5 (*n*=1)	1.8±0.4 (1.5-2; *n*=2)	9±1.1 (1.5-3; *n*=2)	3.3±1.2 (2-5; *n*=8)	3 (*n*=1)	3.3±0.9 (2-4; *n*=8)	4±0.6 (4-5; *n*=3)	
Bar length	23 (*n*=1)	32.5±2.1 (31-34; *n*=2)	8.8±0.7 (15-16; *n*=2)	31±5.2 (20-38; *n*=8)	22 (*n*=1)	24.5±1.9 (22-27; *n*=8)	32±2.1 (30-34; *n*=3)	
Penis length		30.5±0.7 (30-31; *n*=2)	24±2.8 (22-26; *n*=2)	58.2±18.1 (30-73; *n*=5)		27.8±4.3 (24-33; *n*=5)	37 (*n*=1)	30 (*n* = 1)
Accessory Piece length		28±0 (28; *n*=2)	26±1.4 (25-27; *n*=2)	54.2±9.3 (38-60; *n*=5)		32.3±2.9 (28-34; *n*=4)	31 (*n*=1)	24 (*n* = 1)

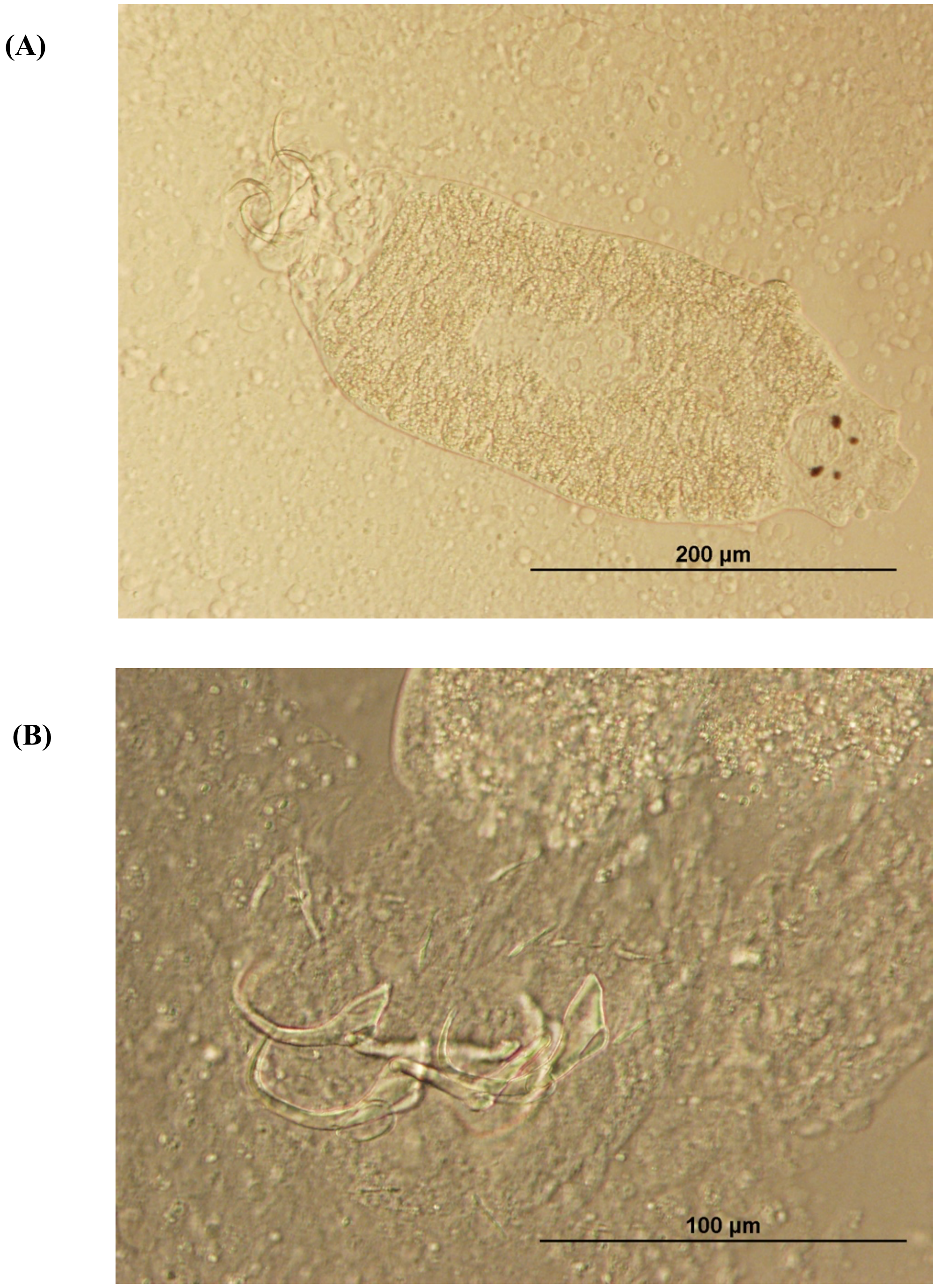


Figure 1. Photographs of *Ligictaluridus pricei* from the gills of *Ameiurus nebulosus* (University of Waikato campus lakes (A) whole body (200x), and (B) posterior (400x).

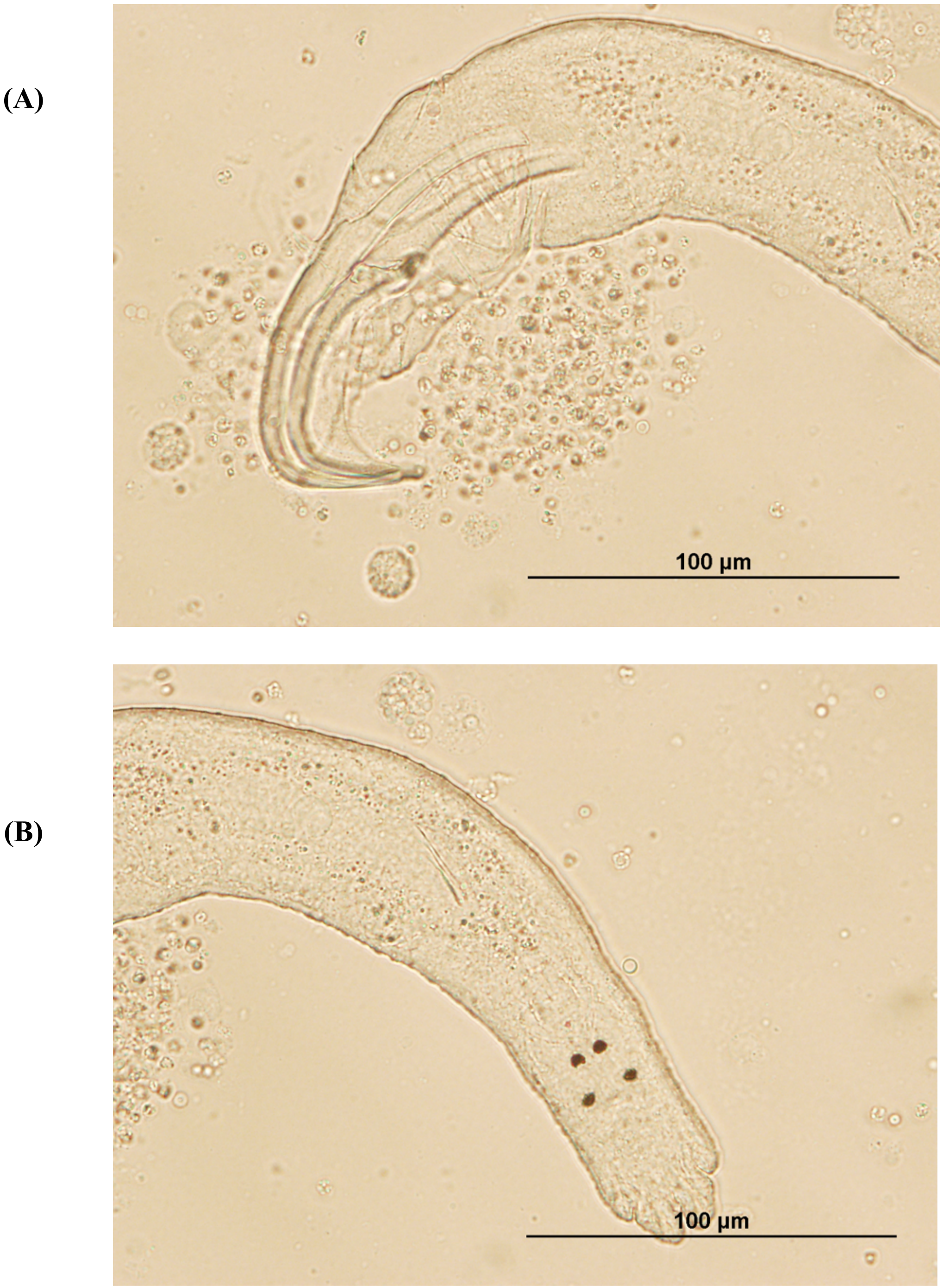
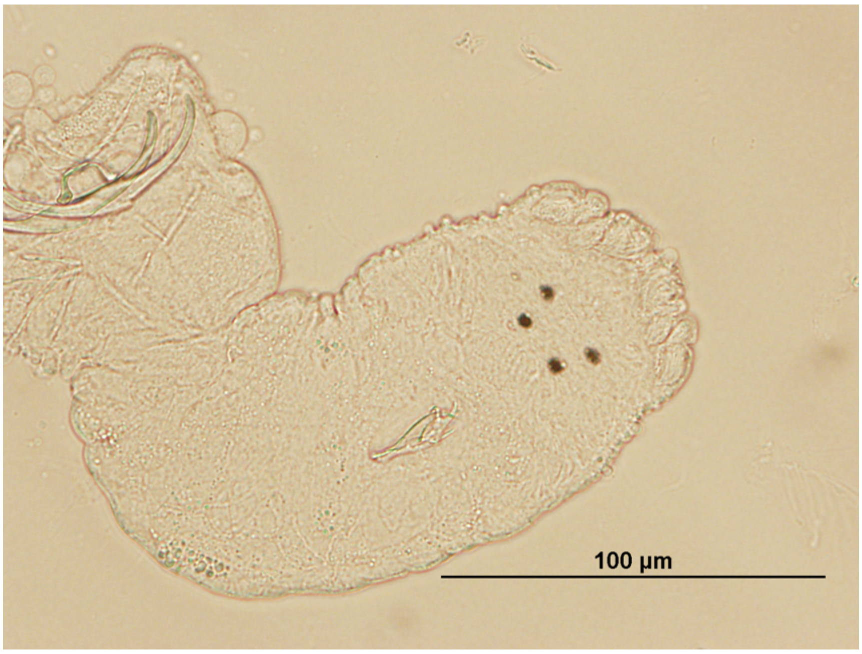


Figure 2. Photographs of *Dactylogyrus anchoratus* from the gills of *Carassius auratus* (University of Waikato Aquatic Research Centre) (A) posterior (400x), and (B) anterior (400x).

Figure 3. Photograph of *Dactylogyrus dulkeiti* from the gills of *Carassius auratus* (University of Waikato Aquatic Research Centre) (400x).

(A)



(B)

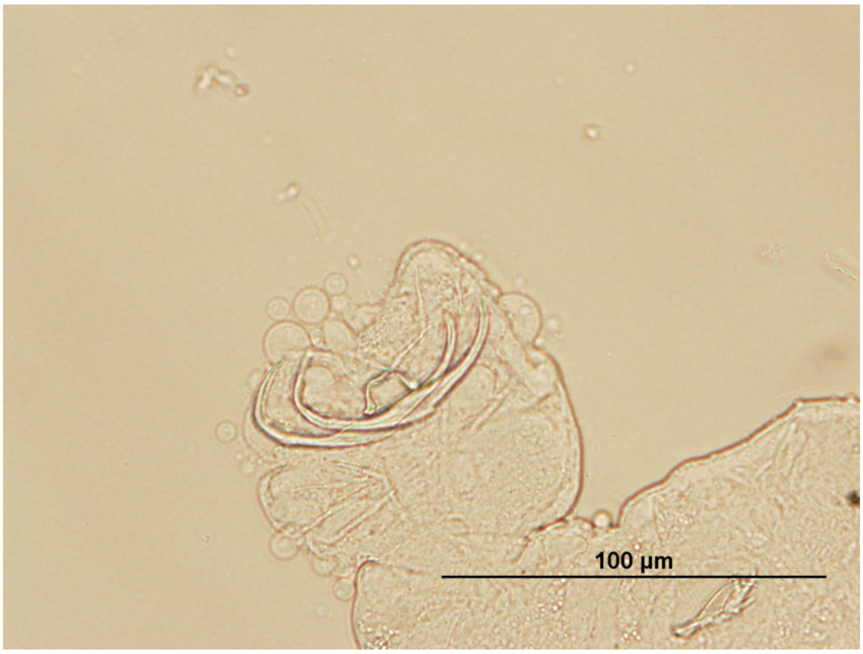


Figure 4. Photographs of *Dactylogyrus formosus* from the gills of *Carassius auratus* (University of Waikato Aquatic Research Centre) (A) whole body (400x) and (B) posterior (400x).

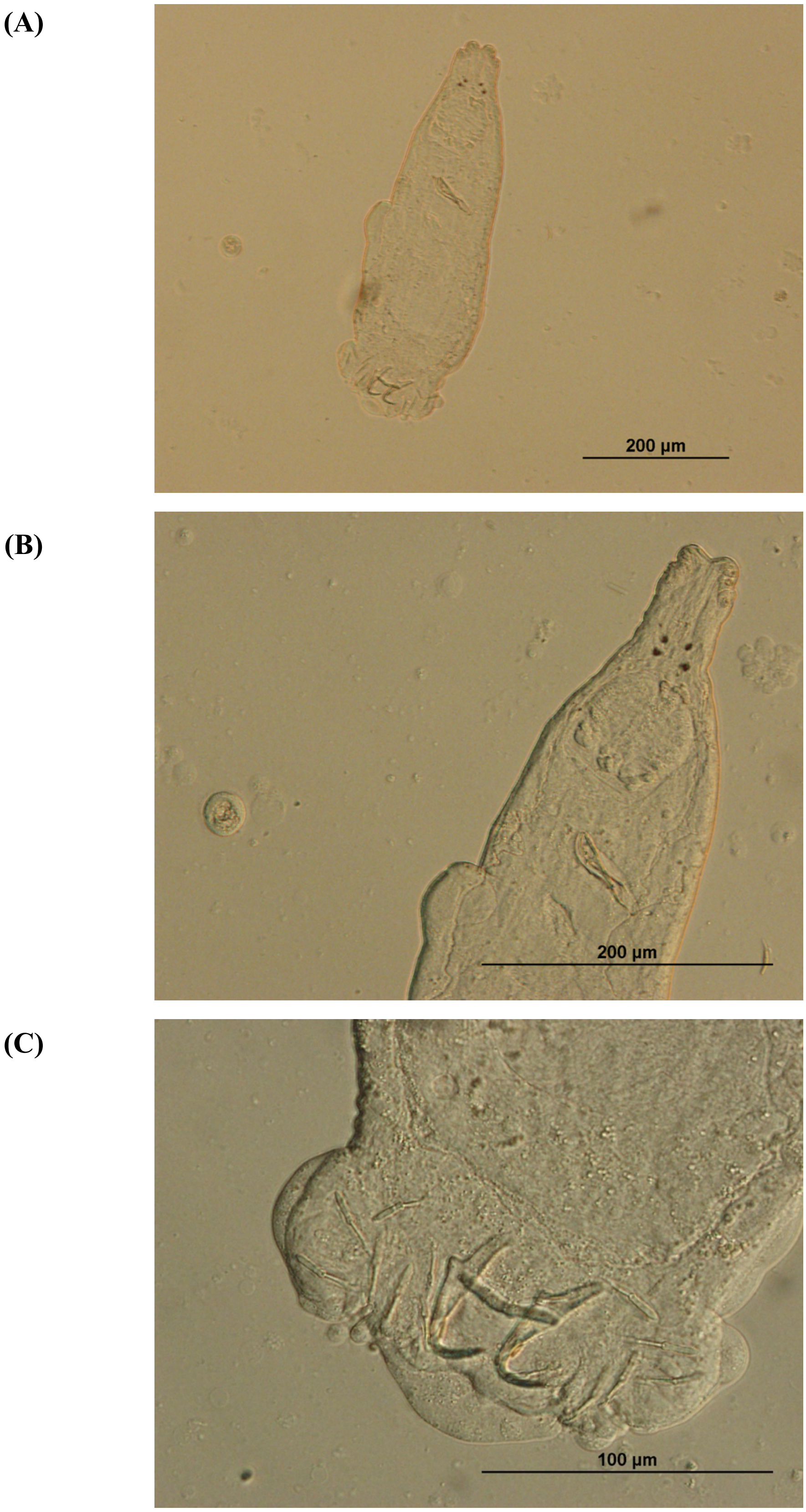


Figure 5. Photographs of *Dactylogyrus inexpectatus* from the gills of *Carassius auratus* (University of Waikato campus lakes) (A) whole body (100x), (B) anterior (200x), and (C) posterior (400x).

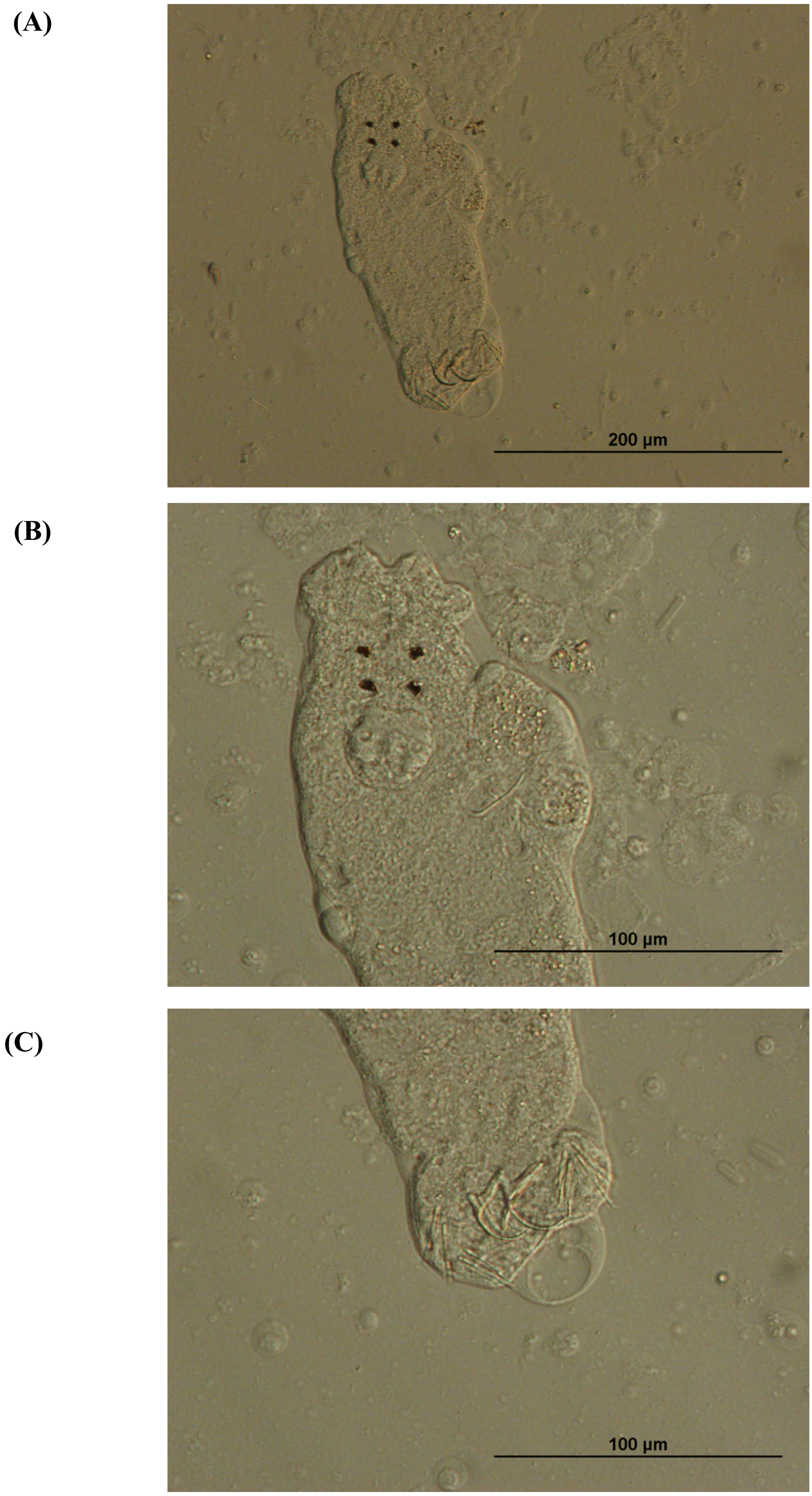


Figure 6. Photographs of *Dactylogyrus intermedius* from the gills of *Carassius auratus* (University of Waikato campus lakes) (A) whole body (200x), (B) anterior (400x), and (C) posterior (400x).

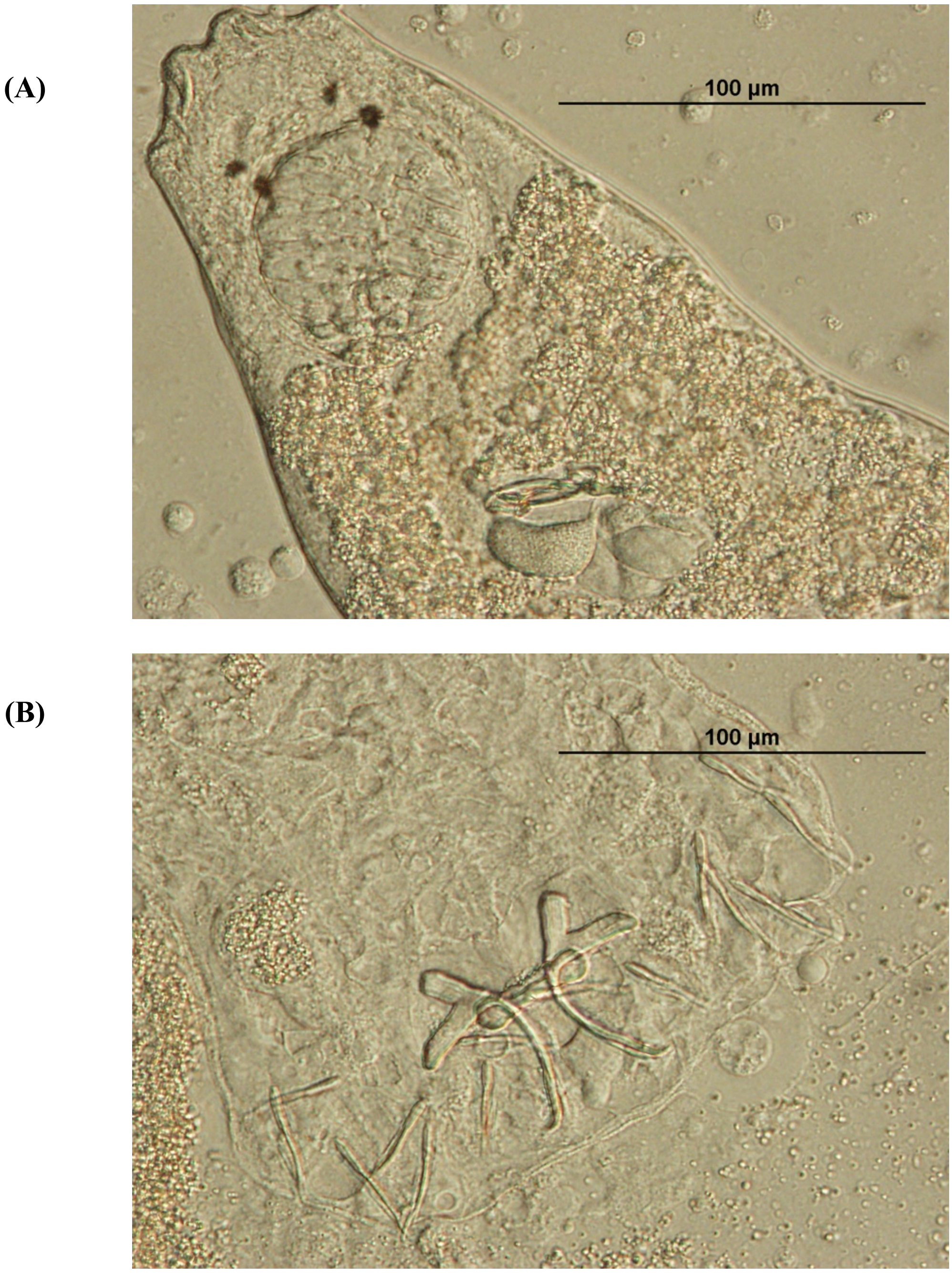


Figure 7. Photographs of *Dactylogyrus vastator* from the gills of *Carassius auratus* (University of Waikato Aquatic Research Centre and campus lakes) (A) anterior (400x), and (B) posterior (400x).

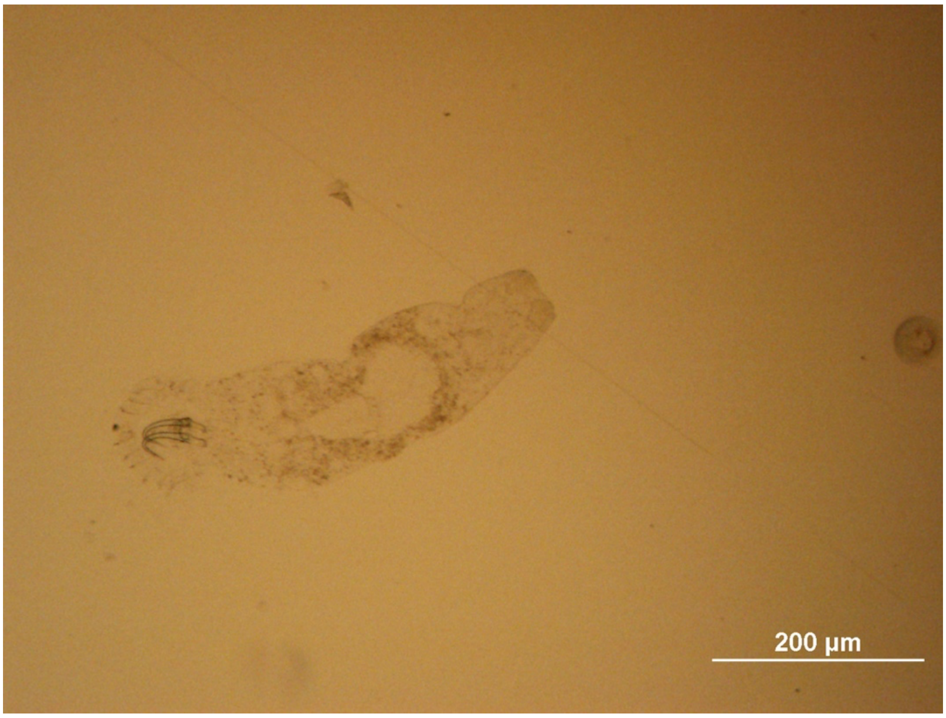


Figure 8. Photograph of *Gyrodactylus* sp. from the gills of *Carassius auratus* (University of Waikato Aquatic Research Centre) (100x).

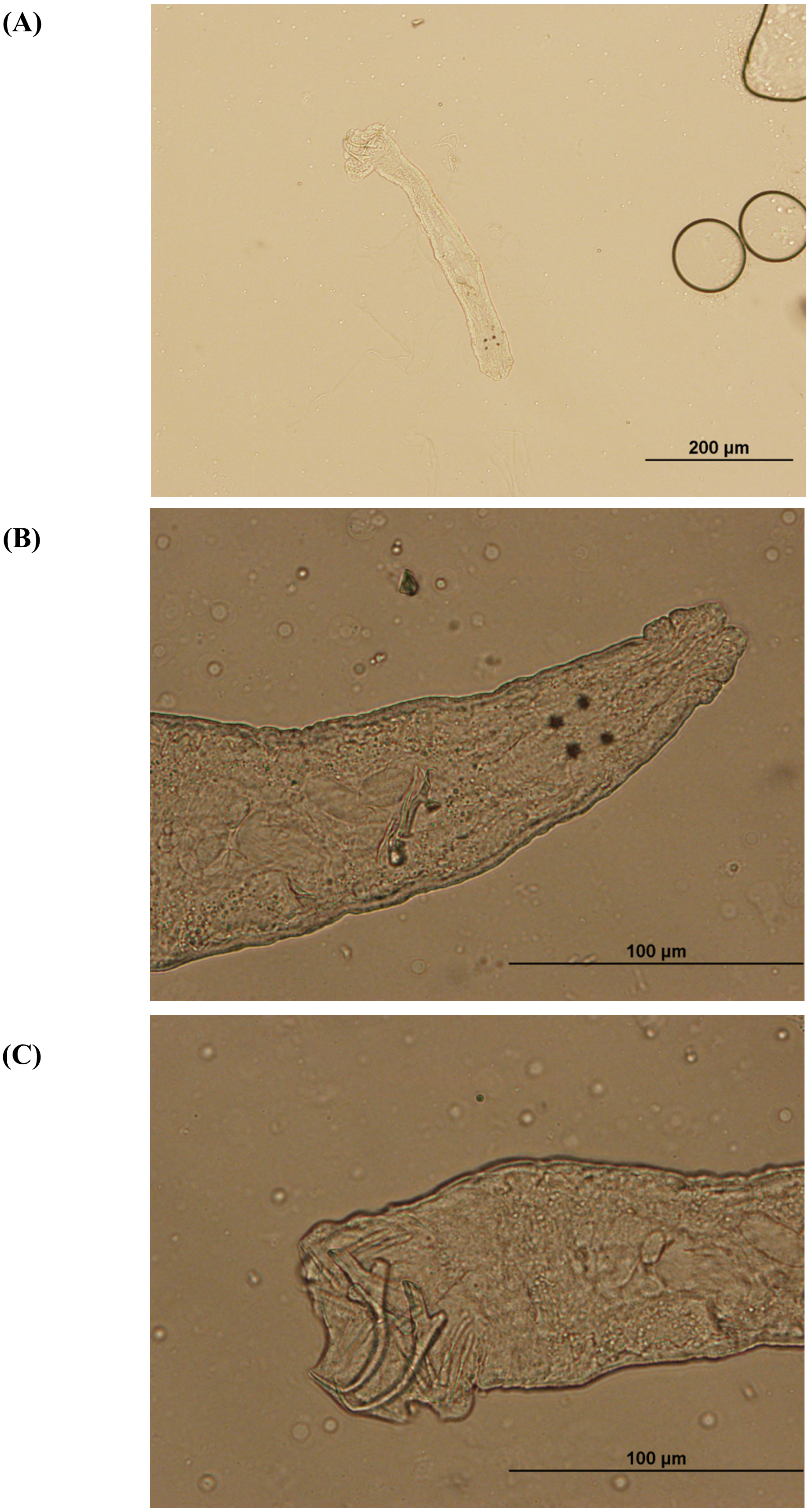


Figure 9. Photographs of *Dactylogyrus minutus* from the gills of *Cyprinus rubrofuscus* (University of Waikato Aquatic Research Centre; Lake Waahi) (A) whole body (100x), (B) anterior (400x), and (C) posterior (400x).

Gambusia affinis was infested with *Salsuginus seculus* (Mizelle & Arcadi, 1945) and *Gyrodactlyus gambusiae* Wellborn & Rogers, 1965. From Lake Ngaroto, 50 *Gambusia affinis* individuals were examined and 40 from Lake Puketirini. The prevalence, mean intensity, and range of intensity are shown in Table 3. A single *Gyrodactylus gambusiae* specimen was found from one *Gambusia affinis* individual, also infested with a single *S. seculus* individual, captured from Lake Puketirini.

Only a single *T. tinca* was examined for monogenean parasites, from which, none were found. Fourteen *S. eryphthothalamus* were examined for monogeneans; again, no parasites were observed. Four *P. fluviatilis* individuals were examined, and none possessed monogeneans. Monogeneans were found infesting the 10 *A. nebulosus* caught from Knighton Lake, but none of the 20 caught from Lake Waahi. Monogeneans were found on the five *Cyprinus rubrofuscus* individuals examined from the laboratory and the two captured from Lake Waahi. Of the nine *Cyprinus rubrofuscus* individuals from Lake Waikare, no monogeneans were observed.

The sixteen juvenile *Carassius auratus* measured 2.4-3.8 cm long and the ten adult *C. auratus*, 21-25.6 cm long. Prevalence of *Dactlyogyrus* in juvenile *C. auratus* was significantly greater than for their adult counterparts ($\chi^2 = 6.64$, df = 1, P = 0.01). Additionally, the mean intensity between these two groups was significantly different (t = 5.98, df = 15.94, 95% CI = 17.09, P<0.001). Values of prevalence and intensity are shown in Table 3.

Table 3. Prevalence and intensity average and range for monogeneans for which these indices could be recorded, from host species collected from different locations.

Host species	Monogenean species	Location	Prevalence	Mean intensity and range
Gambusia affinis ($n = 50$)	*Salsuginus seculus*	Lake Ngaroto	18.0%	2.0 (1-5)
Gambusia affinis ($n = 40$)	*Salsuginus seculus*	Lake Puketirini	42.5%	3.5 (1-21)
Ameiurus nebulosus ($n = 10$)	*Ligictaluridus pricei*	Knighton Lake	54.5%	3.0 (2-6)
Carassius auratus ($n = 15$)	*Dactylogyrus* spp.	Knighton and Oranga Lakes	93.8%	29.7 (10-70)
Carassius auratus ($n = 10$)	*Dactylogyrus* spp.	University of Waikato Aquatic Research Centre	50.0%	1.4 (1-8)

Discussion

The morphologies of the monogenean species identified here were consistent with descriptions and images in literature for both the *Dactylogyrus* species (Pugachev et al., 2009; Tu et al., 2015; Ling et al., 2016; Trujillo-Gonźalez et al., 2018; León et al., 2019; Tancredo & Martins, 2019) and *L. pricei* (Klassen & Beverly-Burton, 1985; Nitta & Nagasawa, 2015; Cloutman et al., 2018; Vancheva et al., 2020). Accompanied by the high host-specificity of monogeneans (Poulin, 1992; Whittington & Chisholm, 2008) this provides a firm basis for the confirmation of the identity of these monogenean species. For the *Gyrodactylus* species from *Carassius auratus*, the morphology was clearly consistent with this genus (Bakke et al., 2007), but only one individual was observed and images could not be obtained that were clear enough to identify to species level.

This brief survey supports the hypothesis of Renner and Duggan (2023); that many previously unreported monogenean taxa may be found in New Zealand on non-native freshwater fish species. It presents entirely new records of eight monogenean species from New Zealand. This survey was, nevertheless, not exhaustive. As many more monogenean species are known from the host species studied elsewhere (e.g., Hoffman 1999), further surveys will undoubtedly identify still more monogenean parasites from non-native fish, especially as many taxa remain unexamined. Three fish species, *P. fluviatilis*, *S. eryphthothalamus*, and *T. tinca*, were not found to be infested with monogeneans. It is still possible that further investigation may find monogeneans infesting these species. In the case of *P. fluviatilis* and *T. tinca*, especially, the absence of monogeneans from this study is likely a result of small sample sizes as only one *T. tinca* and four *P. fluviatilis* were examined. Nevertheless, the possibility of enemy-release (Keane & Crawley, 2002) cannot be discounted entirely. It should be noted that the prevalence of monogeneans on these species has been found to be low on other occasions. For example, in Poland where the prevalence of *Dactylogyrus tincae* (Kathariner, 1895) from *T. tinca* was 3.6% and the prevalence of an unidentified monogenean from *P. fluviatilis* was 3.8% (Kir & Tekin-Özan, 2005). In Turkey the prevalence of *Gyrodactylus medius* (Gusev, 1965) was 0.95% (Mierzejewska et al., 2006). Site may have played a factor in monogenean infestation as all individuals of *S. eryphthothalamus* and three of the four *P. fluviatilis* individuals examined were captured from Lake Waahi. Twenty *A. nebulosus* individuals caught from Lake Waahi were also not infested by monogeneans. This potentially indicates that Lake Waahi represents an environment of poor suitability for monogeneans.

It is possible that time of year may be a factor, also, as monogeneans may experience annual increases and decreases in population size (Chubb, 1977; Blažek et al., 2008; Chapter 2). Nevertheless, no monogeneans were found on *A. nebulosus* from Lake Waahi captured within a week of *A. nebulosus* individuals in Knighton Lake, which had a high prevalence of *L. pricei*. The majority of *A. nebulosus* individuals examined from Lake Waahi were captured at the beginning of May, which may indicate a seasonal explanation for the absence of monogeneans, but as this is only a difference of two months it is difficult to be certain. The *Gambusia affinis* individuals examined from Lake Ngaroto and Lake Puketirini were infested by *S. seculus* and

Gyrodactylus gambusiae which is consistent with those reported by Renner and Duggan (2023) in an examination of *Gambusia affinis* from the University of Waikato campus ponds. No previously unreported monogenean species were found on *G. affinis*. This may be an indicator that the monogeneans of *G. affinis* reported here are a good representation of the species that have been co-introduced into New Zealand. Nevertheless, it is possible that the other known species of monogeneans from *G. affinis* may be found elsewhere in New Zealand, as two other species are known globally (Hoffman, 1999). Sampling the original New Zealand release sites of *G. affinis* in the Auckland Domain and Lake Ngatu, New Zealand, or close to them, may yield other monogenean species. It is notable that the *Cyprinus rubrofuscus* and *Carassius auratus* held at the University of Waikato Aquatic Research Centre were infested with a diverse assemblage of monogeneans. The cyprinid fish had been kept in the tank for at least two years, which would greatly exceed the lifespan of individual monogeneans, which for *Dactylogyrus vastator* is on the order of days to weeks (Vinobaba, 1994). This indicates that these monogenean species have been reproducing in this tank. Five new species records were from fish in this tank, *D. anchoratus*, *D. dulkeiti*, *D. formosus* and *D. vastator* from *Carassius auratus* and *D. minutus* from *Cyprinus rubrofuscus*. *Dactylogyrus vastator* was also observed infesting *Carassius auratus* from the campus ponds. This demonstrates why careful parasitological examination is necessary for aquarium fish as parasites may persist for long periods on or in fish held in captivity.

It is also notable that *C. auratus* had such a high diversity of monogenean species, yielding reports of eight monogenean species whereas other fish species were infested by one or two monogenean species. *Carassius auratus* is known as the host of 44 monogenean species globally (Hoffman, 1999). The related *Cyprinus carpio*, which was until recently synonymised with *C. rubrofuscus* (Xu et al., 2014), is host to 32 species of monogeneans according to Hoffman (1999). *Carassius auratus* and *Cyprinus carpio* represent a rare instance of two host species being infested with several of the same monogenean species (Hoffman, 1999), due to their close taxonomic relationship, having diverged from a common ancestor only 11 Ma ago (Chen et al., 2019). which allows them to hybridise (McDowall, 1990). As *Cyprinus carpio* and *C. rubrofuscus* are even more closely related to one another and were previously synonymised, it may be reasonably assumed that *C. rubrofuscus* is the host of an identical diversity of monogenean species. In this survey, only one monogenean species was reported from *C. rubrofuscus* despite the fact it may be infested with a large number of monogeneans globally similar to *Carassius auratus*. The reason behind the high diversity of monogenean species from *C. auratus* may be due to its popularity as an aquarium fish, which may have led to repeated introductions from a number of locations. In this way, a higher number of monogenean species from this species may have been co-introduced into New Zealand. This study represents one of a small number of studies on New Zealand's freshwater monogeneans and reports the greatest number of freshwater monogenean species in New Zealand, thus far. It indicates a gap in the research of New Zealand aquatic parasites that may continue to be filled.

The difference in *Dactylogyrus* prevalence and intensity between adult *Carassius auratus* which had a prevalence of 50% and mean intensity of 1.4, and juvenile *C. auratus* with a prevalence of 93.8% and a mean intensity of 29.7, is a large one. This supports previous findings that suggest younger fish have greater susceptibility to disease and parasite infection and infestation than their adult counterparts (Ashby & Bruns, 2018; Wunderlich et al., 2022). Although the difference in these parasitological indices between the two age classes was significant, it comes with the caveat of relatively small sample sizes. Overall, 26 *C. auratus* individuals were examined for parasites, 10 of which were adults and 16 were juveniles. Additionally, all but one of the juveniles were caught from the campus ponds at the University of Waikato, Hamilton, and the adult individuals were all captured elsewhere, which leads to the possibility that the differences in parasite numbers observed might be an artefact of fish from the two age groups being sampled from different locations. This is an issue that further research, particularly examining coexisting juvenile and adult *C. auratus* will be able to elucidate. Examining a laboratory population with different age classes of *C. auratus* living together will best help to elucidate an inherent juvenile susceptibility. Despite these caveats, there does appear to be a pattern that warrants further investigation, and it is a finding that is supported by the expectations laid down in previous literature.

These new species records indicate that monogenean parasites of non-native New Zealand freshwater fish have received minimal research. Studies on parasites of New Zealand freshwater fish have recorded other parasite taxa, but monogeneans have not received the same attention (Hine et al., 2000; Zhang, 2012). It seems likely that more monogenean species will yet be reported with further study as there remain many un-surveyed fish species with extant populations in New Zealand and in aquaria. Monogeneans were readily found on the fish they infested and if greater numbers of individuals could be examined for the species that had no monogeneans in the current survey, it is likely to lead to further species being reported. The *Gyrodactylus* species noted here may also yet be identified with a greater taxonomic resolution. Additionally, there will be many native monogeneans on native fish, of which, only one has ever been reported and then, only to the genus level (Hewitt & Hine, 1972). Investigations of native fish will likely reveal native monogenean species that will require description. The significant differences in the monogenean infestations of adult and juvenile *C. auratus* support the hypothesis that younger fish are more susceptible to parasitism, but more targeted research should be undertaken to determine if this pattern is to be found consistently. The number of non-native monogenean species reported here also present opportunities to further study of monogenean ecology in New Zealand.

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Conclusion

In the course of this research, seven of New Zealand's non-native freshwater fish species were examined for monogeneans. From four of these fish species, twelve previously unreported monogenean species were found, eleven of which were identified to species level. From an intensive seasonal survey of western mosquitofish (*G. affinis*), the monogeneans *Salsuginus seculus* from the gills and *Gyrodactylus gambusiae* from the gills and fins, were found as reported in chapter one. Based on these findings it was concluded that many more monogenean species are likely to have established populations in New Zealand, co-introduced with their hosts. Several further monogenean species were found during a survey of non-native New Zealand freshwater fish taxa in chapter three. Firstly, from the gills of brown bullhead catfish (*Ameiurus nebulosus*), *Ligictaluridus pricei* was reported. Both *Salsuginus* and *Ligictaluridus* represent genera new to New Zealand and are from the Ancyrocephalidae, a family that was not previously recognised from New Zealand. From the gills of koi carp (*Cyprinus rubrofuscus*), *Dactylogyrus minutus* was reported. *Dactylogyrus anchoratus*, *D. dulkeiti*, *D. formosus*, *D. inexpectatus*, *D. intermedius* and *D. vastator* were all reported from the gills of goldfish (*Carassius auratus*). These fish species have all had established populations in New Zealand ecosystems for decades, and these new reports highlight the lack of research effort on this taxonomic group in New Zealand. It also suggests that enemy-release may be of little relevance for monogeneans. This case is made strongly for the monogenean species of *Gambusia affinis*, introduced to New Zealand from a Hawaiian population, which in turn was sourced from a Texan population. The retention of these parasites through this process is likely due to the lifecycle of monogeneans requiring no intermediate hosts, allowing a given population of a monogenean species to be sustained anywhere its host species exists.

The study of seasonal population changes in *S. seculus* and *Gyrodactylus gambusiae* over the course of a year in chapter two represents the first study of this nature on freshwater monogeneans in New Zealand, and was indeed, the first examination of freshwater monogenean ecology in New Zealand. Further, although changes in parasite community diversity of *Gambusia affinis* in space and time have been studied before (Carpenter & Herrmann, 2020), this is the first time that seasonal changes in populations in *S. seculus* and *Gyrodactylus gambusiae* have been researched specifically. *Gyrodactylus gambusiae* had far lower abundance than *S. seculus* and was only collected during a handful of months out of the year, which may be a result of differences in reproduction and transmission, but for now, this will remain a problem for future research. The prevalence of *S. seculus* changed significantly over the year, reaching its peak in the summer, with a smaller sharp increase at the end of June (winter). These changes were tested for their correlation to abiotic factors. The concentration of chlorophyll *a* showed the strongest correlation with the prevalence of *S. seculus*, while the pH showed a lesser, but nonetheless significant, correlation. No other measured environmental variable correlated with prevalence, and no measured environmental variable correlated with mean intensity. Notably, the lack of correlation between *S. seculus* population changes and water temperature is a departure from the findings of many previous papers (Chubb, 1977; Valtonen et al., 1990; Ozer & Erdem, 1999; Davidova, 2005; Aydogdu, 2006; Blažek et al., 2008; Wenxiang et al., 2022). The correlations of prevalence to chlorophyll *a* concentration and pH may be because the breeding and population increase of *Gambusia affinis* occurs from late spring to the middle of autumn (Pyke, 2005) and therefore, coincides with annual increases in productivity. Higher concentrations of chlorophyll *a* would indicate higher levels of phytoplankton which would provide more food for grazing zooplankton on which *G. affinis* feed (Pyke, 2005). *Gambusia affinis* are short lived fish, reaching a maximum age of 15 months (Pyke, 2008), and experience large annual seasonal changes in population sizes (Pyke, 2005). Additionally, during breeding the male makes use of an intromittent organ (Pyke, 2005), and the increased proximity of fish brought about by their breeding behaviour may facilitate *S. seculus* transmission. This behaviour, accompanied by annual increases in available hosts for *S. seculus*, likely represent the largest drivers of its population changes. The main conclusion from these results is that seasonal changes in *G. affinis* populations and their breeding patterns are the primary drivers of changes in populations of *S. seculus*, and while environmental factors are of lesser importance.

The relationship between the size of *G. affinis* individuals to the parasitological indices, prevalence and intensity, revealed that the larger fish experienced greater infestation by *S. seculus*, the length being the most significant predictor followed by the weight. Sex was shown to play a role in monogenean infestation in these fish but only by virtue of the fact that the female fish are larger (McDowall, 1990) and therefore had greater levels of infestation. The relevance of size for monogenean infestation is most likely due to the larger gill area available to monogeneans on larger fish, thereby representing a higher quality habitat patch (Kuris et al., 1980). Another possibility is that older fish may have had more time to acquire parasites. This result notably contradicts the hypothesis that younger fish have a greater susceptibility to parasites (Ashby & Bruns, 2018; Wunderlich et al., 2022), potentially making *G. affinis* an unusual case. Internal parasite taxa may reduce the ability of fish hosts to defend against further parasites and their presence would contribute to the measured weight of fish (Timi & Poulin, 2020). This may contribute to the higher prevalence and intensity of *S. seculus* on heavier fish. In *Carassius auratus*, the much smaller individuals were observed to have a significantly higher overall monogenean prevalence and intensity than adults, supporting the view that younger fish are more vulnerable to parasites and disease (Ashby & Bruns, 2018; Wunderlich et al., 2022). This observation did, however, come with the caveat of a small sample size and the fact that the adult and juvenile *C. auratus* were not living in association with one another. Despite this, juvenile *C. auratus* having a *Dactylogyrus* prevalence of almost 100% and intensities reaching higher than 50, there appears to be a pattern here. Such a pattern is given credence by previous research and warrants further investigation by examining coexisting age classes in these fish using greater sample sizes of fish.

The clearest direction for further research on freshwater monogeneans in New Zealand would be the examination of further fish species. Seven different non-native fish species were examined in this research, representing one-third of the non-native freshwater fish species with extant populations in New Zealand. By examining more fish species in New Zealand, it is highly likely that more further monogenean species will be identified. Three of the fish species examined in this study, *S. erythrophthalamus*, *P. fluviatilis*, and *T. tinca* were not infested with monogeneans, though monogenean species are known from these fish species elsewhere in their native and invaded ranges (Hoffman, 1999). Should these species be examined in greater numbers in future, they may yield monogenean parasites also. Additionally, by examining more fish across New Zealand it will help to determine the distribution of their monogeneans. *Ameiurus nebulosus* individuals caught from Lake Waahi were not infested by monogeneans but those sampled from the University of Waikato Hamilton campus ponds had a high prevalence of the monogenean *Ligictaluridus pricei*. This may be a matter of uneven distribution of this monogenean species, or it may be that Lake Waahi represents an unsuitable habitat for these monogeneans. Both of these represent questions that may be tested in future research. While records of species are valuable, there remains more to be understood about the ecology and effects of the organisms themselves. My study of the seasonal changes in a population of *S. seculus* may provide a basis for further investigation into seasonal changes in monogenean populations in New Zealand. In temperate continental countries where many seasonal studies of monogeneans have often been undertaken, there are large annual changes in temperature (Duckson, 1987) and many researchers have concluded that temperature is a significant driver of monogenean population changes (Chubb, 1977; Valtonen et al., 1990; Ozer & Erdem, 1999; Davidova, 2005; Aydogdu, 2006; Blažek et al., 2008; Wenxiang et al., 2022). The link between temperature and monogenean population changes is a reasonable one, as temperature is known to affect the development of monogeneans (Gelnar, 1987; Zhang et al., 2022). Observing population changes in monogeneans in a locality with a mild climate has to a degree controlled for this commonly reported link between temperature and monogenean population cycles. Unlike the tropics, where annual temperatures remain largely constant and monogenean populations do not change greatly between the wet and dry seasons, New Zealand experiences four seasons, albeit with a narrower annual temperature range than in continental climates. The commonly seen pattern of monogenean population increases over the summer has occurred here but without an evident direct link to the temperature. I suggest that the most important driver of seasonal population changes in *S. seculus* are the population changes and breeding behaviour of *G. affinis*. How the seasonal changes in populations of monogenean species on other fish species may be influenced is, however, another question. By utilising the mild climate of New Zealand, the temperature influence has been mitigated allowing the seasonal changes in an *S. seculus* population to be related instead to biotic factors. With several new monogenean species records on multiple fish species, further studies of this nature may be carried out in the unique climate of New Zealand. The short lifespan and seasonal population changes of *G. affinis* appears to provide good explanations for the population changes in *S. seculus*, but in fish species with longer lifespans the situation may be somewhat different. If such a study were conducted on monogenean parasites of *C. auratus* or *Ameiurus nebulosus*, fish with lifespans considerably longer than *G. affinis*, and different modes of reproduction (McDowall 1990), would significant annual changes in monogenean populations still occur? And if so, what might cause those changes? Perhaps these fish, lacking similar life histories to *G. affinis*, would not experience significant changes of monogenean populations throughout the year, or they may experience changes at different times, perhaps coinciding with the breeding activity of their hosts.

Other aspects of monogenean ecology from these fish could also be examined. For instance, the impact of host size differences in monogenean infestation, which were highly relevant for

G. affinis. Future research could examine this in other fish species and determine if it would represent a trend for multiple species. There was a major difference in the two age classes of *C. auratus* examined here, but they were taken from different locations and a relatively small number were examined. However, this result does represent a preliminary finding which could be built upon by future research. The low numbers observed of *Gyrodactylus gambusiae* may be of particular interest in future research. It is difficult to estimate why this may have been the case. *Gyrodactylus gambusiae* was found in low numbers or was entirely absent in the University of Waikato Hamilton campus ponds on all sampling dates. Additionally, of 40 *Gambusia affinis* samples from Lake Puketirini, only one *Gyrodactylus gambusiae* individual was observed, indicating that this species may have low abundance generally. This hypothesis is supported by the lack of literature on the species and a low prevalence and abundance of what is presumed to be *G. gambusaie* from Texas (Carpenter & Herrmann, 2020). It might be that these monogenean species rarely make attempts to transmit from once host to another as this is generally a risky manoeuvre for *Gyrodactylus* individuals (Bakke et al., 2007). *Carassius auratus* examined in this research were infested with many species of monogeneans though not all coexisted on a single fish individual simultaneously. This raises the question as to why *C. auratus* apparently possesses the highest diversity of monogeneans of the non-native New Zealand freshwater fish species examined. Both *Carassius* and *Cyprinus* species are hosts of many monogenean species elsewhere; 44 species from *Carassius auratus* and 32 from *Cyprinus carpio* (Hoffman, 1999). Further, these two fish species are infested by many of the same monogenean species. Of the monogenean species reported here, *D. anchoratus*, *D. formosus*, *D. vastator* and *D. minutus* are found on both *Cyprinus carpio* and *Carassius auratus* elsewhere. Given their close taxonomic relation, *Cyprinus rubrofuscus* is likely infested with the same monogenean species as *C. carpio*. It is possible that the higher diversity of monogenean species reported from *Carassius auratus* is due to their popularity as an aquarium fish which may have led to several introductions from illegal releases. If *Cyrpinus rubrofuscus* and *Carassius auratus* share the four monogenean species mentioned above, it raises the question as to why none of these species were found on both fish species. The answer to this question may result from competition among monogenean species. Such a high diversity of monogeneans on this fish species would provide a New Zealand researcher with the opportunity to study monogenean community ecology, including how different monogenean species interact with each other. The possibilities for studies of monogenean communities in New Zealand has been expanded with several new species records and the first ecological work on this class of parasites in this country.

There has been little research on freshwater monogeneans in New Zealand previously. One parasite checklist for New Zealand fishes (Hine et al., 2000) demonstrates this well; it lists numerous species of various parasite taxa, including 19 freshwater Digenea and 10 freshwater Cestoda, but only three freshwater monogenean species. The monogeneans reported in the present study were readily observed on the fish examined, potentially indicating a bias in previous parasitological investigations. It seems unlikely that these monogeneans could have been otherwise overlooked. Possibly, the focus of other research has been on the endoparasites of fish and their outer surfaces have not been adequately examined. Monogeneans may not easily be seen as they are small animals, with those species reported here ranging from approximately 200 to 500 µm in length, so a dedicated examination for monogeneans on fish surfaces is required to observe them. If such an examination has been lacking in any general surveys of parasites previously, then it may explain why more species reports have not been made. The fact that no researcher has attempted to focus on monogeneans specifically, remains a mystery.

Overall, the results of my thesis have demonstrated that little research effort has been undertaken on freshwater monogeneans in New Zealand in the past, and this thesis thus represents pioneering work on the understanding of their ecology in this country.

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