Digenean larvae in *Hydrobia ulvae* from Belfast Lough (Northern Ireland) and the Ythan Estuary (north-east Scotland)

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In this study the digenean parasites in the mud snail *Hydrobia ulvae* at two sites were compared for a period of one year. The sites were the north shore of Belfast Lough (Northern Ireland) and the Bend of Tarty on the Ythan Estuary (north-east Scotland). Samples of snails were collected monthly from which 13 species of digenean larvae were recorded at Belfast Lough. Two of these species were absent at the Bend of Tarty. Overall digenean prevalence was very similar at each site indicating that the respective bird communities were composed of ecologically equivalent species. Differences in the prevalence of individual digenean species are discussed in relation to the abundance of definitive and/or secondary intermediate hosts at each of the sites. Both sites displayed similar seasonal patterns of parasite prevalence with sharp increases to around 25% in the summer months. Snail shell heights were greater at Belfast Lough than at the Bend of Tarty and a positive correlation between snail shell height and infection by trematode parasites was recorded at both sites. Increased parasite prevalence in larger snails was attributed to factors such as age-related susceptibility and/or parasitic castration of the snails.

INTRODUCTION

The estuaries and sea loughs of the British Isles support enormous numbers of migrant and resident birds, the vast majority of which are infested by parasites. Many of the parasites are digeneans and nearly all species use a gastropod mollusc as a first intermediate host. It is not surprising therefore that gastropod populations in areas where birds congregate possess a high prevalence of larval digeneans. One such gastropod is the mud snail *Hydrobia ulvae* which occurs in great densities on most of the muddy sea loughs and estuaries of northern Europe.

In this study the digenean parasites occurring in *H. ulvae* at two sites were compared in terms of their species and prevalence for the period of one year. One site was on the north shore of Belfast Lough in Northern Ireland, (map reference IGR-J 355810) close to the University of Ulster at Jordanstown. The other was at the Bend of Tarty on the Ythan Estuary (map reference NGR-NJ 996273) at Newburgh, Aberdeenshire. Both sites are sheltered tidal mudflats on the east coast of a land mass and they support large populations of waders and seabirds. The trematode fauna on Belfast Lough has been the subject of numerous investigations (Irwin, 1997) and the flora and fauna of the Ythan are considered to be the best documented in the world (Gorman & Raffaelli, 1993).

The Ythan Estuary supports internationally important numbers of breeding sandwich terns (*Sterna sandvicensis*) (Pritchard et al., 1992), as well as nationally important flocks of breeding eiders (*Somateria mollissima*), common tern (*Sterna hirundo*) and little tern (*Sterna albifrons*) and

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also wintering eider and redshank (*Tringa totanus*). Belfast Lough is of international importance for wintering redshank and turnstone (*Arenaria interpres*) and of national importance for wintering great crested grebes (*Podiceps cristatus*), goldeneye (*Bucephala clangula*), oystercatcher (*Haematopus ostralegus*), scaup (*Aythya marila*) and curlew (*Numenius arquata*) (Pritchard et al., 1992).

There are many differences between the sites, not least that the Ythan is an estuary whereas the site chosen on Belfast Lough is much more marine and quite distant from a major freshwater influence. The Belfast Lough site is in an urban area which is frequently disturbed by baitdiggers and walkers. It is also exposed to a range of nearby industrial activities including land reclamation, refuse tipping, sewage disposal and dredging. The Bend of Tarty is much more rural and less disturbed. It is subject to little pollution except agriculture fertilizer leachate. Most of the Ythan's shorebirds feed on the mudflats and mussel beds during low tide. On the mudflats the main prey items are small invertebrates such as Corophium volutator, Hydrobia ulvae, Nereis diversicolor and Macoma balthica, all of which can occur in extremely high densities (Anderson, 1971; Gorman & Raffaelli, 1993). The estuary is a highly diverse system containing a variety of fish, shrimps, crabs, annelids and molluscs, all of which contribute to its productivity. In contrast, the north shore of Belfast Lough has a low species diversity and a high species richness. The polychaetes, N. diversicolor and Capitella capitata, the amphipod Corophium volutator and the gastropod H. ulvae are particularly well represented (MarEnCo, 1992).

MATERIALS AND METHODS

Each month, from January to December 1994 (inclusive), samples of the mud snail populations at the Bend of Tarty and the north shore of Belfast Lough were taken along predetermined line transects, which ranged from high water neap tide to low water neap tide. On both shores the transects were about 80 m in length. Samples of mud were collected at 0.5 m intervals along the transects to a depth of 4 cm. These were taken until a container measuring 25×25×4 cm was filled. The mud was sieved through a 1-mm mesh and the remaining material retained. Following transportation to the University of Ulster, it was placed in white sorting trays and covered with filtered seawater. The central area of each tray was cleared of debris and mud, allowing snails to crawl into the cleared patch for random selection. The tray was repeatedly shaken and each time a new central area was cleared. This procedure was repeated until 400 snails were collected from each sample. The snails were placed in fresh, filtered seawater and maintained at 4°C until the length of each could be measured to the nearest 0.1 mm using callipers. Snails were crushed and the presence of digenean larvae was recorded. They were identified using an identification key by Deblock (1980).

While collecting the March and October samples, 6 quadrats of mud measuring $25 \times 25 \times 4$ cm deep were taken. This mud was washed though a 1-mm sieve and the remaining debris retained. It was treated in the same manner as the other samples except that all the *Hydrobia ulvae* present were collected and counted to allow the total number of snails per m² of mud to be estimated.

The data accumulated over the one year sampling period were analysed and manipulated using a Statview 512+ package on an Apple Macintosh computer.

RESULTS

Species from six families of Digenea were found in the samples collected from the Bend of Tarty (Table 1). Five of these families, the Echinostomatidae, Heterophyidae, Psilostomatidae, Notocotylidae and Haploporidae, were represented by one species whereas a total of six species belonged to the Microphallidae. Of the 11 digenean species observed, eight occurred as cercariae. The remaining three species, *Maritrema oocysta, Microphallus abortivus* and *Levinseniella* sp. no. 17, were metacercarial cysts located in the gonad of the snails. Only six species observed have complete life cycle descriptions. Microphallid sp. no. 15, *M. abortivus, Levinseniella* sp. no. 17, Notocotylidae sp. no. 12 and Gymnophallid sp. no. 4 have been described only as the larval stages found in *Hydrobia ulvae*.

Data accumulated from the Belfast Lough samples revealed seven digenean families in *H. ulvae*, with a total of 13 species identified. In the case of the Microphallidae, Echinostomatidae, Psilostomatidae, Notocotylidae and Haploporidae, the parasite species observed from the Belfast Lough and the Bend of Tarty samples were the same. *Cryptocotyle jejuna*, a heterophyid, was also common to both sites. Two species, *Bunocotyle progenetica* and *Pygidiopsis ardeae* were observed only in samples collected from Belfast Lough. The family Microphallidae, with six species, was by far the best represented in snails from both sites.

Of the 4800 snails collected from the Bend of Tarty, 649 (13.5%) were infected with larval digeneans. A total of 604 (12.6%) infected snails were observed in the 4800 collected from the north shore of Belfast Lough during the same period. When these were compared using statistical analysis no significant difference in trematode prevalence between the sites was observed (χ^2 =1.935, P=0.1642).

In order to assess if a similar pattern of parasite prevalence existed for both sample sites a null hypothesis of no difference in prevalence levels between the Bend of Tarty and the north shore of Belfast Lough was adopted. When a two-sample Kolmogorov–Smirnov test was applied to the data the result indicated that both the level and development in prevalence at each site was similar.

Although prevalences for the majority of parasite species in the snail populations from each site were low,

Table 1. Digeneans identified in Hydrobia ulvae from the north shore of Belfast Lough and the Bend of Tarty, along with the prevalence of the eight most common species and the significance (χ^2 -test) of differences (ns, not significant). Overall parasite prevalences are also stated.

Family	Species	Belfast Lough % prevalence	Bend of Tarty % prevalence	Р
Microphallidae	Microphallid sp. no. 15	4.64	2.54	0.001
•	Maritrema oocysta	0.98	4.50	0.001
	Microphallus primas	1.54	1.18	0.02
	Levinseniella brachysoma	0.48	0.34	ns
	Microphallus abortivus	0.54	0.21	0.01
	Levinseniella sp. no. 17	Present	Present	
Heterophyidae	Pygidiopsus ardae	Present	Not present	
- /	Cryptocotyle jejuna	1.60	1.44	ns
Haploporidae	Gymnophallid sp. no. 4	Present	Present	
Echinostomatidae	Himasthla continua	1.98	1.60	ns
Psilostomatidae	Psilostomum brevicolle	Present	Present	
Notocotylidae	Notocotylidae sp. no. 12	0.06	0.81	0.001
Hemiuridae	Bunocotyle progenetica	Present	Not present	
Overall prevalence	2 1 6	12.6	13.5	ns

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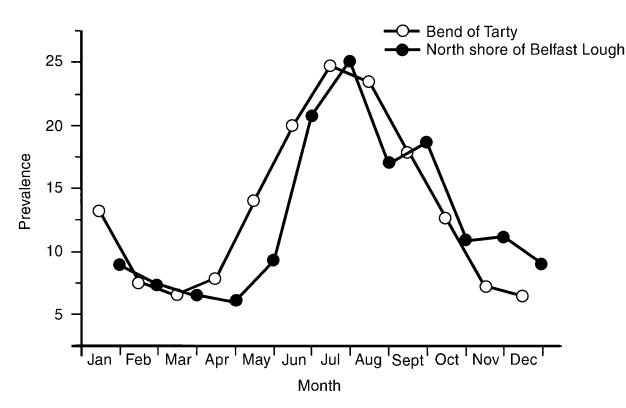


Figure 1. Parasite prevalence at the Bend of Tarty and the north shore of Belfast Lough over the 12 month period.

there were differences between those occurring at the Bend of Tarty and the north shore of Belfast Lough (Table 1). Comparison of the prevalence of eight of the most common parasite species shows that four, Himasthla continua, Levinseniella brachysoma, Microphallus primas and C. jejuna occurred with similar frequency at both the Bend of Tarty and the Belfast Lough site. On the other hand Microphallid sp. no. 15 and Microphallus abortivus, an encysted metacercaria which has only been identified as the larval form, appeared almost twice as frequently in the Belfast Lough samples as in those from the Bend of Tarty. The encysted metacercariae of Maritrema oocysta was four times more frequently encountered in samples from the Bend of Tarty than in snails from Belfast Lough. Notocotylidae sp. no. 12 was infrequently found in samples from Belfast Lough, with approximately 93% of the total observations being in samples from the Bend of Tarty.

When infection levels were examined over the one year period, both sample sites displayed seasonal fluctuations in parasite prevalence (Figure 1). The Bend of Tarty sample site exhibited a monomodal curve with low prevalence during February, March and April and a sharp increase during May and June to reach a peak in July of 24.8%. It then decreased over the following months to 6.5% in December. Similarly, parasite prevalence in samples from Belfast Lough was also low during February, March and April rising in May and June to 25.5% in July. However the prevalences in Belfast Lough started to decrease in August only to rise slightly in September, decreasing slowly to reach a low of 9% in December.

Analysis of snail densities recorded during March and September showed there were 4031 snails m^{-2} (SD=215) at the Belfast Lough site compared to 6107 snails m^{-2} (SD=216) at the Bend of Tarty.

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Table 2. Shell height (to nearest 0.1mm) of uninfected and infected Hydrobia ulvae from each site (N=4800).

	Bend of Tarty	Belfast Lough
Uninfected H. ulvae		
Mean height	3.9	4.2
Range	2.0 - 6.2	2.1 - 7.5
Most frequently encountered		
height	4.0	4.6
Infected H. ulvae		
Mean height	4.3	4.8
Range	2.6 - 6.2	2.9 - 7.5
Most frequently encountered		
height	4.0	4.9

Shell heights of the sampled population at each site are displayed in Table 2 and they show that, on average, the snails from Belfast Lough were larger than those from the Bend of Tarty. When only infected snails are compared (Table 2) a similar pattern is revealed. A significant difference (P < 0.001) was also observed when the size of infected and uninfected snails from both sites were compared. It indicated a positive correlation between snail shell height and infection by digenean parasites.

DISCUSSION

When comparing the results obtained in this study with those provided by others, it must be remembered that different sampling procedures may have been adopted. However, it is noteworthy that Anderson (1971), in his study of the biology of *Hydrobia ulvae* in the Ythan Estuary, recorded 6% prevalence with digeneans. Huxham et al. (1995) recorded a very similar figure for the overall prevalence in *H. ulvae* at the Ythan but that result was a composite derived from five sampling stations around the estuary which had prevalences ranging from 1.5 to 16.8%. The highest prevalence (16.8%) recorded by Huxham et al. (1995) was for the Bend of Tarty and that compares very closely to the 13.5% average prevalence recorded in this study for the same area.

A comparison of the results of this study and a similar one of digenean parasitism in *H. ulvae* from the north shore of Belfast Lough also reveals a consistency of results. Saville (1992), who took samples from the same area and adopted a very similar sampling procedure to the one utilized in this study, recorded that 11.7% *H. ulvae* contained trematode parasites. That result compares favourably with the 12.6% recorded in this study.

Perhaps the similarity in the levels of parasitism at the two sites can be largely explained by similarities in the bird communities at the sites. It is known that most parasites can adapt to, and utilize, a variety of hosts (Holmes, 1979; Esch & Ferndandez, 1993) and consequently, avian hosts which consume the same food organisms are most likely to harbour the same parasites. It would seem therefore that the bird communities at each of the two sites must comprise species with similar ecological niches. This does not infer that the species are taxonomically similar. Rather it would appear that the two sites support parallel communities composed of ecologically equivalent species.

Huxham et al. (1995) identified eight digenean species in *H. ulvae* from the Ythan Estuary. Three of these species, Notocotylidae sp. no. 14, Himasthla interrupta and Maritrema subdolum were not recorded in the present study. However, five species recorded in this study, Pygidiopsis ardeae, Psilostomum brevicolle, Bunocotyle progenetica, Notocotylidae sp. no. 12 and Gymnophallid sp. no. 4 were not observed by Huxham et al. (1995). The differences in parasite species observed may be due to the different sampling methods employed. Huxham et al. (1995) took samples randomly from the mudflat whereas, in this study, a transect was used and samples were collected from various stations along its length. It is possible that certain parasites are more frequently found at particular levels on a mudflat depending on the feeding behaviour of their definitive hosts. According to Ankel (1962) a rough correlation exists between the bathymetrical distribution of first intermediate hosts and the type of definitive host, with some parasites more common on the upper shore than mid or lower shore, though there is usually some overlap and no distinct area can be categorized for a particular parasite species. Annual variation in presence or absence of definitive hosts would also have an effect on the presence of larval stages in Hydrobia ulvae.

All four parasite species (*Himasthla continua*, *Levinseniella brachysoma*, *Microphallus primas* and *Cryptocotyle jejuna*) which were found in similar numbers at both sites, have had their life cycles fully described at some time in the scientific literature. In each case, these parasites were in an environment which supplied the appropriate second intermediate hosts and definitive hosts required for their maintenance. For example, *L. brachysoma* is released from *H. ulvae* and encysts in *Corophium volutator*, which is commonly found in large numbers at both sample sites.

The definitive hosts of this parasite include shelduck (*Tadorna tadorna*), turnstones and oystercatchers (Mac-Donald, 1969) and these were observed frequently at both sites. With each host available in substantial numbers it is not surprising that this parasite is commonly observed at the Bend of Tarty and the north shore of Belfast Lough. Similarly *M. primas*, released from *H. ulvae* as an active cercaria, would also find a plentiful supply of second intermediate hosts, *Carcinus maenas* (Saville & Irwin, 1991) and their definitive host, oystercatchers (MacDonald, 1969) at both sites.

Although the list of digeneans was very similar at the two sites, Bunocotyle progenetica and Pygidiopsus ardeae were observed only in samples from Belfast Lough. Pygidiopsus ardeae was observed only three times and, given this level of rarity, it is possible that it was present at the Bend of Tarty but not in the samples of snails collected. It may also be significant that this parasite, which is naturally found in grey herons (Ardea cinerea) (Køie, 1990), was absent from the Bend of Tarty as herons were never seen while sampling was being carried out at this site. On the other hand B. progenetica, which was isolated six times in samples from Belfast Lough, does not use an avian definitive host but is found in the soft tissue under the pyloric stomach of mullet (Mugil sp.) (Overstreet, 1977). It is unlikely that these fish are found in the Ythan Estuary because of its northerly location. Mullet prefer warmer climates and they do not appear in a list of species found in the Ythan Estuary (Hall & Raffaelli, 1991). This may explain the absence of B. progenetica infections in H. ulvae from the Bend of Tarty.

Microphallid sp. no. 15, Microphallus abortivus, Maritrema oocysta and Notocotylidae sp. no. 12 were more frequently observed at one or other of the sample sites. Little is known about these organisms and only M. oocysta has a complete life cycle description. According to Deblock (1980) the definitive host for this species is the redshank. Available information on bird numbers reveals that redshank appear to be more common on the Ythan Estuary. This might explain why *M. oocysta* was four times more common in the Bend of Tarty samples. Microphallid sp. no. 15 and *M. abortivus* were more frequently observed in samples from the north shore of Belfast Lough. It must therefore be assumed that some suitable definitive hosts are frequenting this area. Perhaps their higher levels of prevalence may be due to the greater number of gulls feeding and roosting on the north shore of Belfast Lough, which is adjacent to the Belfast City Council refuse dump. Gulls are known to harbour a large variety of parasites (MacDonald, 1969), reflecting the broad nature of their diet and the fact that they are opportunist feeders. As they are attracted in large numbers to the nearby refuse dump it would not be surprising if they were responsible for many of the digenean larvae in the snails collected from the north shore of Belfast Lough.

Notocotylidae sp. no. 12 was more frequently observed in samples from the Bend of Tarty. It is possible that this particular species is more common there because of the larger numbers of anseriformes present at that site (Pritchard et al., 1992). According to Deblock (1980) members of the Notocotylidae are frequently found in ducks, though it is possible that this parasite could utilize other bird species as its definitive host. The parasite fauna in *H. ulvae* from the Bend of Tarty and the north shore of Belfast Lough fluctuated over time, a phenomenon widely reported in other invertebrates including *Littorina littorea* (L.) (Robson & Williams, 1970) and *Cerithidea californica* (Yoshino, 1975; Snyder & Esch, 1993). Temporal variation of digenean prevalence in snail hosts is frequently related to the behaviour of definitive hosts, snail growth patterns and environmental factors (Kuris, 1990; Mouritsen et al., 1997). All three of these factors may have influenced the pattern observed at the Bend of Tarty and further work would be needed to ascertain the effect of each.

The monomodal seasonal prevalence observed at the Bend of Tarty could have been the consequence of the snail reproductive cycle and feeding activity. Anderson (1971) who examined the breeding cycle of *H. ulvae* on the Ythan Estuary, found that although capsules containing snail eggs were observed every month of the year, the main spawning season was April-July with a peak in May. Increase in parasite prevalence therefore occurred after the snails had reproduced. This agrees with earlier findings that snails are more susceptible to parasite invasion after reproduction (Lauckner, 1986). Secondly, increased snail grazing associated with higher summer temperatures might also enhance the possibility of snails becoming infected as they would be more likely to come into contact with fluke eggs in the sediment (Fernandez & Esch, 1991). The snails might also be more likely to be invaded by miracidia which had been stimulated to hatch by the higher summer temperatures (Anderson, 1974).

This study has provided some important and novel information on the trematode parasite population of H. *ulvae* at the two geographically separated sites. According to Esch & Fernandez (1993), many more long-term studies on parasites and their hosts are required before predictions about patterns of parasitism can be made.

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REFERENCES

- Anderson, A., 1971. Intertidal activity, breeding and the floating activity of *Hydrobia ulvae* in the Ythan Estuary. *Journal of the Marine Biology Association of the United Kingdom*, **51**, 423–437.
- Anderson, R.M., 1974. Population dynamics of the cestode Caryophyllaeus laticeps (Pallas, 1781) in the bream Abramis brama (L.). Journal of Animal Ecology, 43, 305–321.
- Ankel, F., 1962. Hydrobia ulvae (Pennant) und Hydrobia ventrosa (Montagu) als Wirte larvaler Trematoden. Videnskabelige Medderelelser fra Dansksk Naturhorisk Forening, 124, 1–100.
- Deblock, S., 1980. Inventaire des trématodes larvaires parasites des mollusques *Hydrobia* (Prosobranches) des côtes de France. *Parassitologia*, 22, 1–105.
- Esch, G.W. & Fernandez, J.C., 1993. A functional biology of parasitism. London: Chapman & Hall.
- Fernandez, J.C. & Esch, G.W., 1991. The component structure of larval trematodes in the pulmonate snail *Helisoma anceps*. *Journal of Parasitology*, **77**, 540–550.

Gorman, M. & Raffaelli, D., 1993. The Ythan Estuary. *Biologist*, **40**, 10–13.

- Hall, S.J. & Raffaelli, D., 1991. Food-web patterns: lessons from a species-rich web. *Journal of Animal Ecology*, **60**, 823–842.
- Holmes, J.C., 1979. Parasite populations and host community structure. In *Host-parasite interfaces*. (ed. B.B. Nickol), pp. 27–46. New York: Academic Press.
- Huxham, M., Raffaelli, D. & Pike, A.W., 1995. The effect of larval trematodes on the growth and burrowing behaviour of *Hydrobia ulvae* (Gastropoda: Prosobranchia) in the Ythan Estuary, north-east Scotland. *Journal of Experimental Marine Biology and Ecology*, 185, 1–17.
- Irwin, S.W.B., 1997. Excystation and cultivation of trematodes. In Advances in trematode biology (ed. B. Fried and T.K. Graczyk), pp. 57–86. Boca Raton: CRC Press.
- Køie, M., 1990. Pygidiopsis ardeae n. sp. (Digenea: Heterophyidae: Pygidiopsinae) in the grey heron Ardea cinerea L. from Denmark. Systematic Parasitology, 15, 141–149.
- Kuris, A., 1990. Guild structure of larval trematodes in molluscan hosts: prevalence; dominance and significance of competition. In *Parasite communities: patterns and processes* (ed. G.W. Esch et al.), pp. 69–100. London: Chapman & Hall.
- Lauckner, G., 1986. Ecological effects of larval trematode infestations on littoral marine invertebrate populations. *International Journal for Parasitology*, **17**, 391–398.
- MarEnCo Ltd., 1992. Inner Belfast lough area of special scientific interest: monitoring programme. Belfast: MarEnCo Ltd.
- MacDonald, M.E., 1969. Catalogue of helminths of waterfowl (Anatidae). Washington, DC: Bureau of Sport Fisheries & Wildlife. [Special Scientific Report no. 126.]
- Mouritsen, K.N., Jensen, T. & Jensen, K.T., 1997. Parasites on an intertidal *Corophium*-bed: factors determining the phenology of microphallid trematodes in the intermediate host populations of the mud-snail *Hydrobia ulvae* and the amphipod *Corophium volutator. Hydrobiologia*, **335**, 61–70.
- Overstreet, R.B., 1977. A revision of Saturnius Manter, 1969 (Hemiuridae: Bunocotylinae) with descriptions of two new species from the striped mullet. Instituto de Biologia publicaciones especiales, 4, 273–284. [Excerta Parsitologica en memoiria del docteur Eduardo Caballero.]
- Pritchard, D.E., Housden, S.D. Mudge, G.P. & Galbraith, C.A., ed., 1992. Important bird areas in the United Kingdom including the Channel Island and the Isle of Man. Slimbridge: Royal Society for the Protection of Birds.
- Robson, E.M. & Williams, I.C., 1970. Relationships of some species of Digenea with the marine prosobranch *Littorina littorea* (L.). I. The occurrence of larval Digenea in *Littorina littorea* on the North Yorkshire coast. *Journal of Helminthology*, 44, 153–168.
- Saville, D.H., 1992. Studies on the developmental stages of a marine parasite Microphallus primas (Digenea: Microphallidae). PhD thesis, University of Ulster, Northern Ireland.
- Saville, D.H. & Irwin, S.W.B., 1991. In ovo cultivation of Microphallus primas (Trematoda: Microphallidae) metacercariae to ovigerous adults and the establishment of the life cycle in the laboratory. Parasitology, 103, 479–484.
- Snyder, S.D. & Esch, G.W., 1993. Trematode community structure in the pulmonate snail *Physa gyrina*. *Journal of Parasitology*, 79, 205–215.
- Yoshino, T.P., 1975. A seasonal and histological study of larval Digenea infecting *Cerithidea californica* (Gastropoda: Prosobranchia) from Goleta Slough, Santa Barbara County, California. *Veliger*, 18, 156–161.

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