ASSESSMENT OF ENDEMIC TOOTHCARP (*APHANIUS DANFORDII*) AND INVASIVE MOSQUITOFISH (*GAMBUSIA HOLBROOKI*) HEALTH BY MEANS OF RELATIVE CONDITION FACTOR UNDER THE CO-INFECTION OF DIFFERENT PARASITE GROUPS AND REVELATION OF THE EFFECTS ON THEIR COMPETITIVE INTERACTIONS

Ahmet ÖZER[a,*], Emel ÇANKAYA[b], Türkay ÖZTÜRK[a]

*aozer@sinop.edu.tr

[a]*Sinop University, Faculty of Fisheries and Aquatic Sciences, 57000 Sinop, Turkey*
[b]*Sinop University, Faculty of Science and Letters, Department of Statistics, Sinop, Turkey*

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1. Introduction

Fish serve as hosts to ecto- and endoparasites that exhibit a wide variety of lifecycle strategies,

Parasites can reduce growth, fecundity and survival, and change behaviour and sexual characteristics of the infected fish hosts,

The host–parasite relationship is often unbalanced, and parasites can severely compromise the health of their hosts and even kill them,

Most of the studies showing the effects of parasites on wild fish populations have been carried out in freshwater ecosystems and parasites on their host fishes reported to be causing either direct mortality or indirect effects such as decline in recruitment success and growth, alteration on host behaviour, reduction in swimming speed and increased risk of predation,

However, the effects of parasites on the health status of endemic and introduced fish populations, the relationship between parasitism, condition, reproduction, and natural mortality of these species remains largely unknown and as a consequence, parasites are often overlooked in fish health assessments, Studies on the effects of parasites on the health condition of fishes are very rare,

Fish condition can be extremely important to fisheries management as plump fish may be indicators of favourable environmental conditions (e.g., habitat conditions, ample prey availability), whereas thin fish may indicate less favourable environmental conditions,

The relative condition factor (Kn), one of the commonly applied condition factor used to determine the effects of parasites on their hosts in natural populations, corresponds to the ratio between observed and theoretically expected weight for a given length and allows statistical comparison between the estimated value of Kn and the central value Kn = 1.0,

The relative condition factor Kn greater than one (1) is indicative of the general well being of fish, whereas its value less than one (1) indicates that fish is not in a good condition.

Figure 1. *Gambusia holbrooki*

Figure 2. *Aphanius danfordii*
M: Male F: Female

Gambusia holbrooki is a species of freshwater fish and is a member of the family Poecilidae of order Cyprinodontiformes. They feed readily on the aquatic larval and pupal stages of mosquitoes and are called as eastern mosquitofish. It is also thought that this fish has potential to cause damage to endemic fish and other endemic aquatic life.

Aphanius danfordii is also a species of freshwater fish and is a member of the family Cyprinodontidae of order Cyprinodontiformes and is called as killifish. This fish is fed on crustaceans, insect larvae and some plankton species and has a potential of controlling mosquitoes.

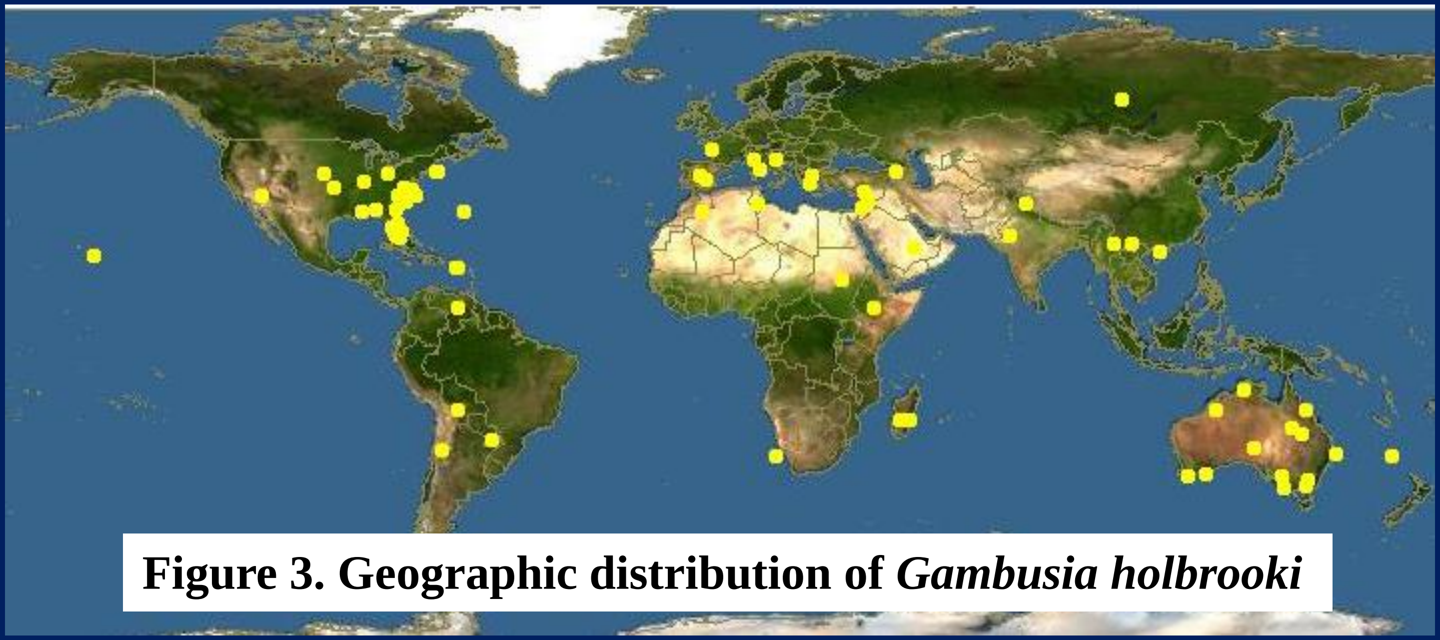

Figure 3. Geographic distribution of *Gambusia holbrooki*

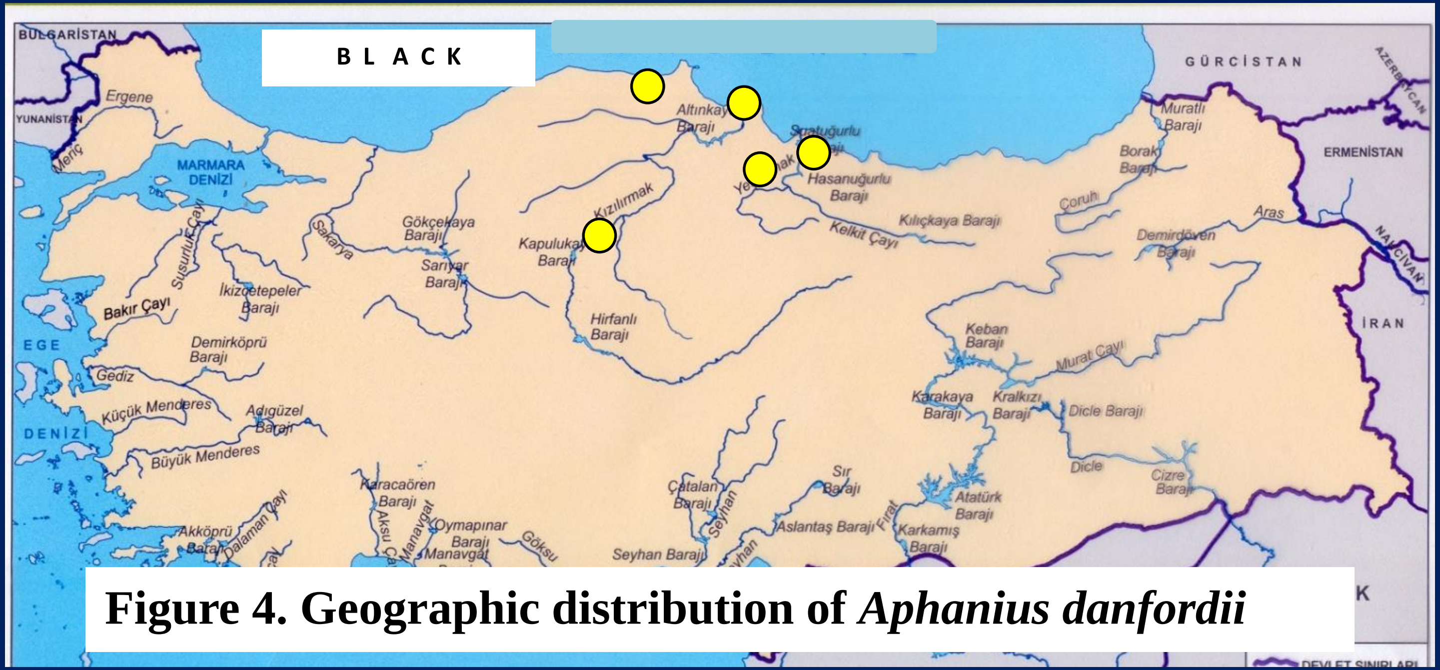

Figure 4. Geographic distribution of *Aphanius danfordii*

2. Materials and Methods

Fish from Lower Kızılırmak Delta (Figure 5) were collected by electroshock device in the period between 15 December 2010 and 14 November 2011.

We measured whole weight (0.1 g) and total length (0.1 cm).

All fish underwent a systematic external and internal examination in tissues including skin, fins, gills, eyes (lens and vitreous humour), body cavity and visceral organs (stomach, intestine, liver, swimbladder, heart and gonads).

Parasites recovered were fixed and preserved using methods commonly applied. Identified parasites were grouped as follow: (1) Protozoa, (2) Monogenea, (3) Digenea, (4) Acanthocephala, (5) Copepoda, (6) Cestoda, (7) Nematoda and used for comparing the effects on fish health status at higher taxonomic level.

Calculation of infection prevalence (%), mean intensity and mean abundance follow Bush et al. (1997). Briefly, prevalence (%) is the percentage of infected fish while mean intensity is the average number of parasites in the total number of infected fish and abundance is the average number of parasites in the total number of examined fish.

Figure 5. Map of sampling area in Turkey

Weight-Length Relationship (WLR):

The relationship between the measurements of length and weight of fish was modelled by means of the parabolic equation (Froese, 2006):

$$W = a L^b$$

where

W = weight of fish (in grams),

L = length of fish (in centimetres),

a = initial growth coefficient

b = an exponential term of growth.

The logarithmic form of this model was used to convert this relationship into the straight-line relationship and then the estimation of parameters *a* and *b* was achieved by robust regression modelling with *log*(a) = intercept and b=slope terms.

Relative Condition Factor (Kn):

The theoretically expected weight for a given length was calculated by using the estimated weight-length relationship curve. Then the relative condition factor (Kn) corresponds to the ratio of observed weight (W) and expected weight (W_e) as below (LeCren, 1951):

$$K_n = \frac{W}{W_e}$$

In order to compare the condition, fatness or well-being of fish, based on the assumption that heavier fish of a given length are in better condition, the mean ***Kn*** values were calculated for the groups of fish infected by:

- **a)** different parasite groups (e.g. with Trichodina) and the non-parasitized group (e.g. without Trichodina) independently of all the others present
- **b)** each particular parasite group *alone* and all combinations (co-infection) of different parasite groups

A statistical comparison of these mean values with the standard value ***Kn***=1 was performed by the Student-t test. Besides, test of equality of mean weight as well as length measurements between the groups defined in (*a*) was performed by the Student-t or Mann Whitney-U test.

Effect of Season, Number of Parasite Groups and their Interaction:

Regardless of the parasite groups, seasonal effect and parasite load on both fish species' health was separately examined by comparing the mean *Kn* values obtained for each season and also for the groups of fish bearing different number of parasite groups to the hypothesized standard value of 1.

Student-t test was performed for this purpose. Additionally, interactive effect of the season and number of parasite groups on the host were examined graphically and tested statistically by Kruskal Wallis test.

3.Results

Table 1. Parasite species identified from both fish species throughout the examination period.

Aphanius danfordii	***Gambusia holbrooki***
Trichodina domerguei (Figure 6)	*Trichodina* sp. (Figure 10)
Trichodina modesta (Figure 6)	
Trichodina heterodentata (Figure 6)	
Tripartiella macrosoma (Figure 6)	
Vorticella sp. (Figure 6)	*Vorticella* sp. (Figure 10)
Gyrodactylus sp. (Figure 7)	*Gyrodactylus* sp.
Salsiginus sp. (Figure 7)	-
Ascocotyle sp. (Figure 8)	*Echinostoma* sp. (Figure 10)
Diplostomum spathaecum (Figure 8)	*Diplostomum spathaecum* (Figure 10)
Tylodelphys clavata (Figure 8)	*Tylodelphys clavata* (Figure 10)
Posthodiplostomum sp. (Figure 8)	*Posthodiplostomum* sp. (Figure 10)
Unidentified digenea mtc. (Figure 8)	-
Neoechinorhynchus rutili (Figure 8)	-
Spiroxys contortus (Figure 9)	-
Hysterothylacium aduncum (Figure 9)	-
Schulmanella petrichowski (Figure 9)	*Schulmanella petrichowski* (Figure 11)
Bothriocephalus acheilognathi (Figure 6)	*Bothriocephalus acheilognathi* (Figure 10)
Ergasilus sieboldi	-

Table 2. Identified parasite groups with species and their overall infection prevalence (%) and mean intensity values in toothcarp (*Aphanius*) and eastern mosquitofish (*Gambusia*) in the Lower Kızılırmak Delta throughout the sampling period (S.E.=Standard Error).

Parasite species	Prevalence (%)		Mean Intensity ± S.E.	
	Aphanius	*Gambusia*	*Aphanius*	*Gambusia*
PROTOZOA	58.2	8.3	372.5 ± 98.5	7.1 ± 4.2
MONOGENEA	29.6	0.6	4.3 ± 0.7	1.0 ± 0.0
DIGENEA	99.2	69.6	93.3 ± 13.6	15.1 ± 2.8
CESTODA	0.8	10.6	1.0 ± 0.0	2.0 ± 0.3
NEMATODA	8.8	10.1	1.6 ± 0.3	5.7 ± 1.4
ACANTHOCEPHALA	4.0	-	1.0 ± 0.0	-
COPEPODA	10.4	-	2.0 ± 0.3	-

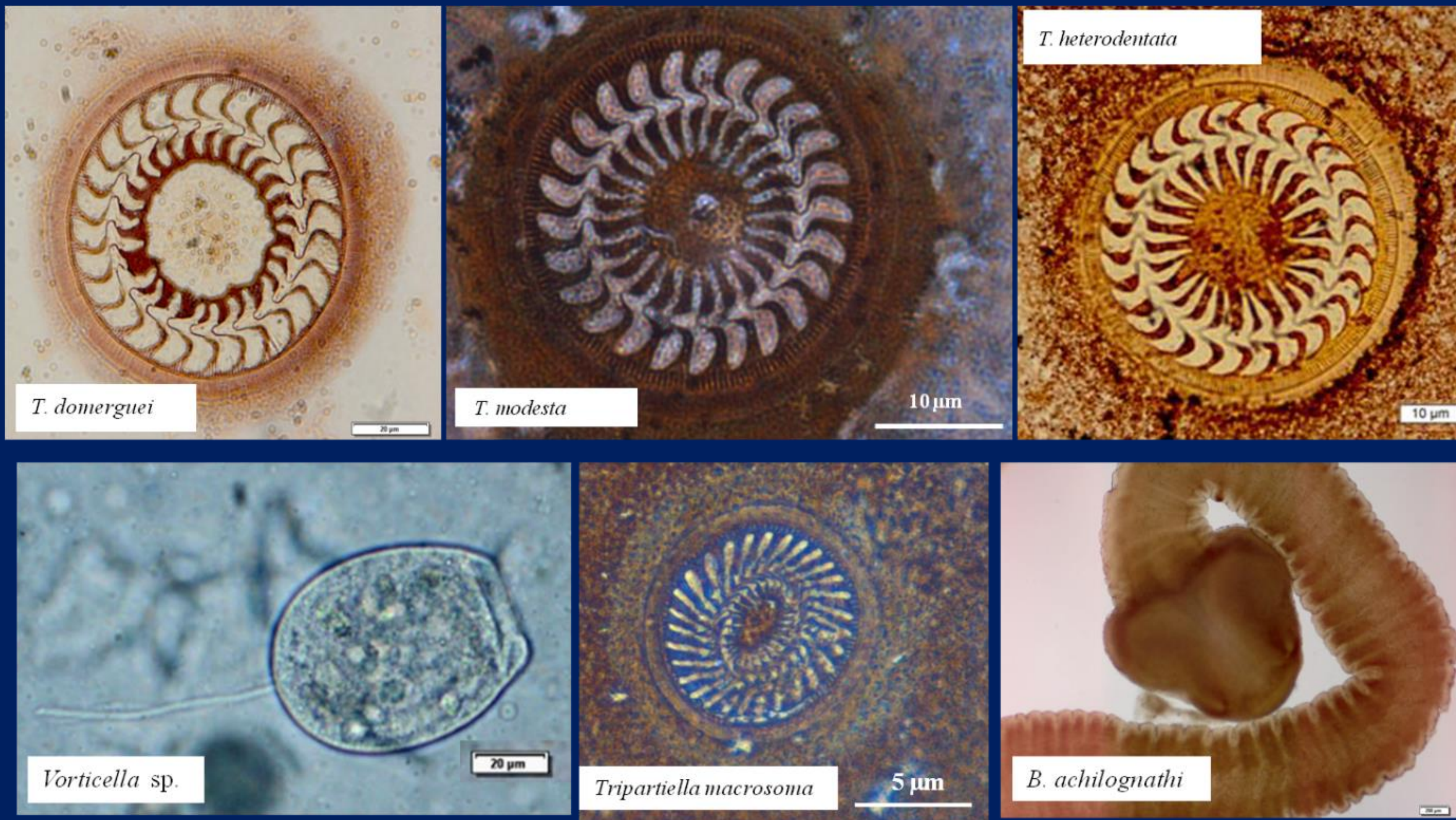

Figure 6. Identified trichodinid, vorticella and cestoda parasites in toothcarp.

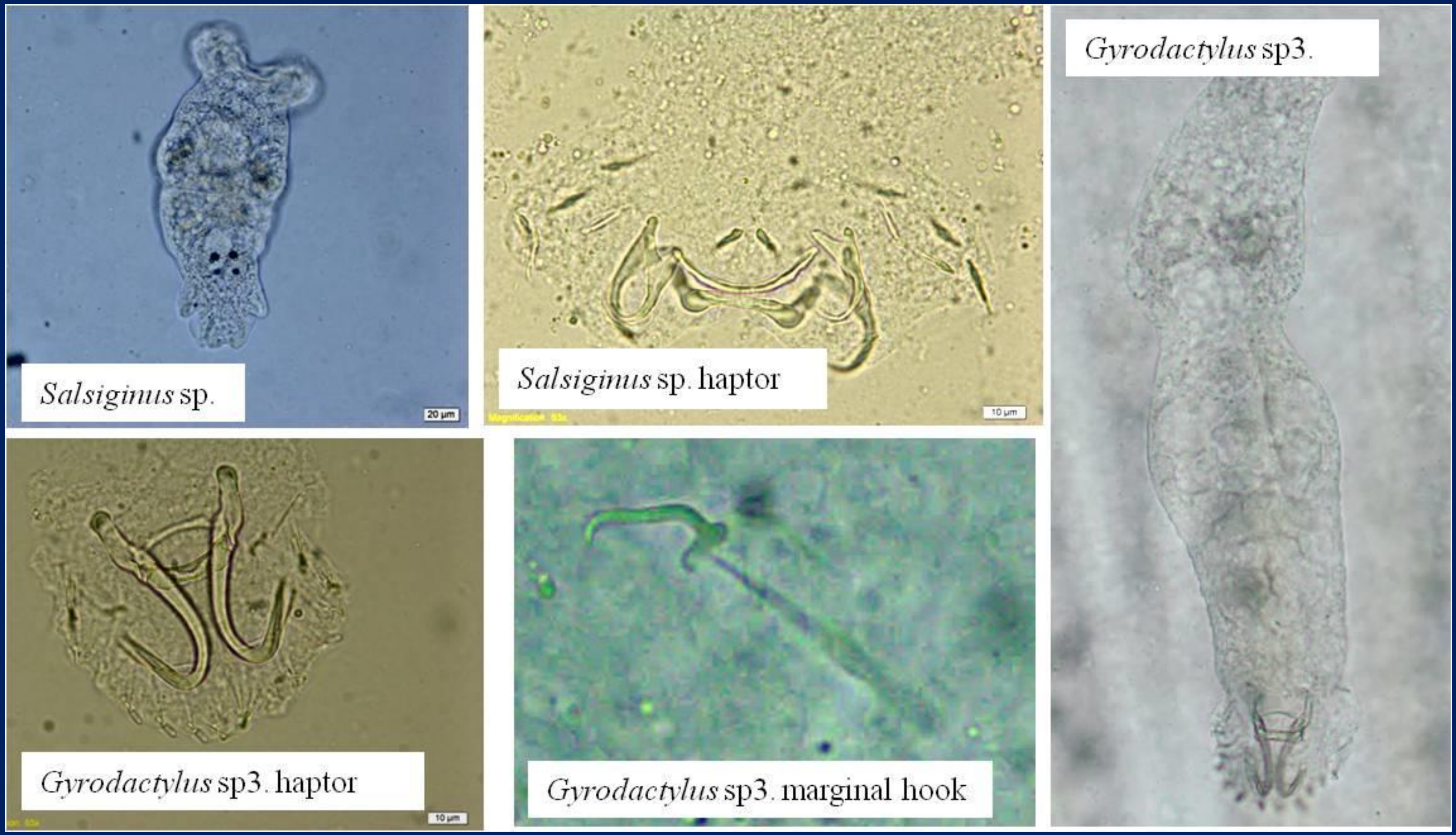

Figure 7. Identified monogenean parasites in toothcarp.

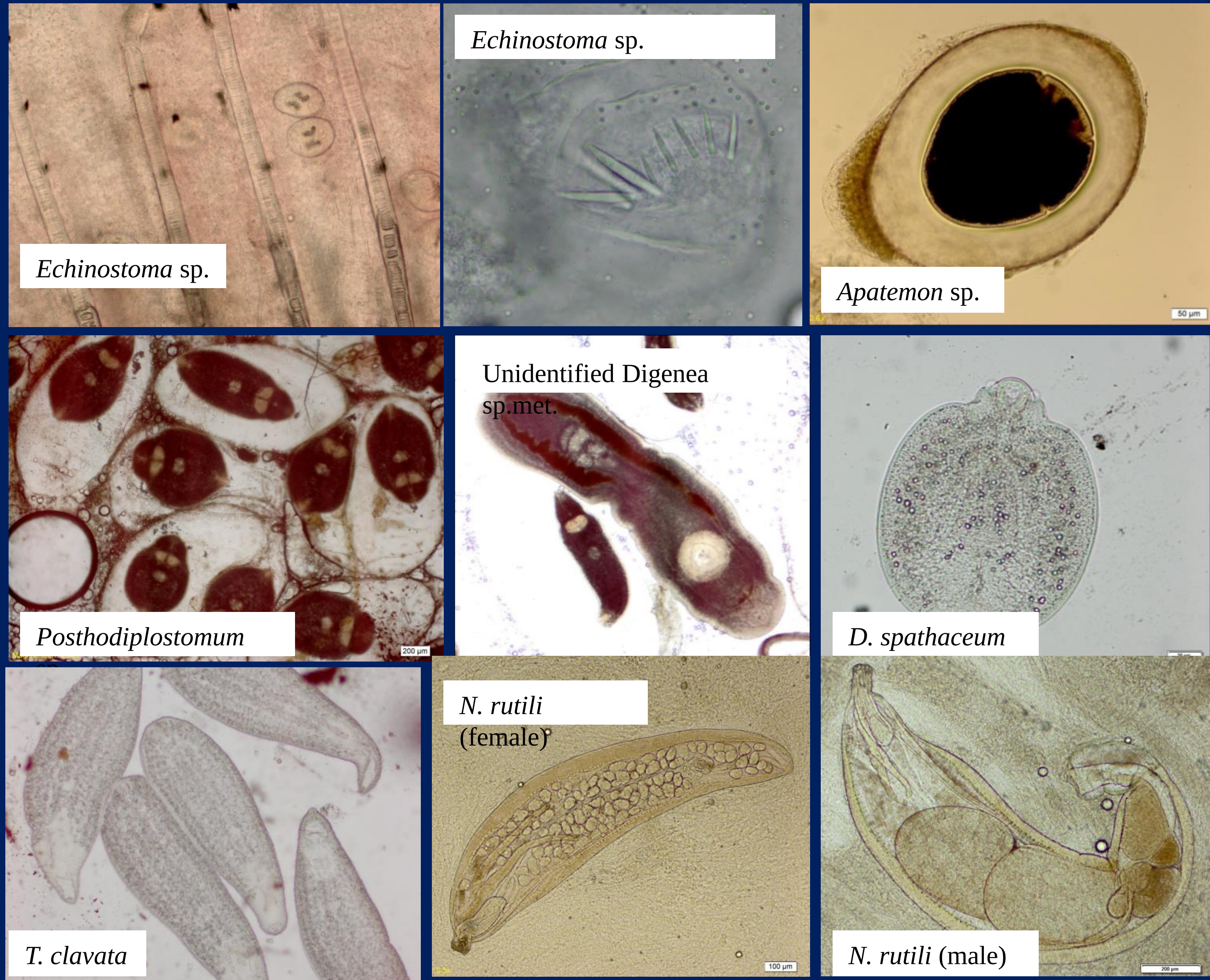

Figure 8. Identified digenean and acanthocephala parasites in toothcarp.

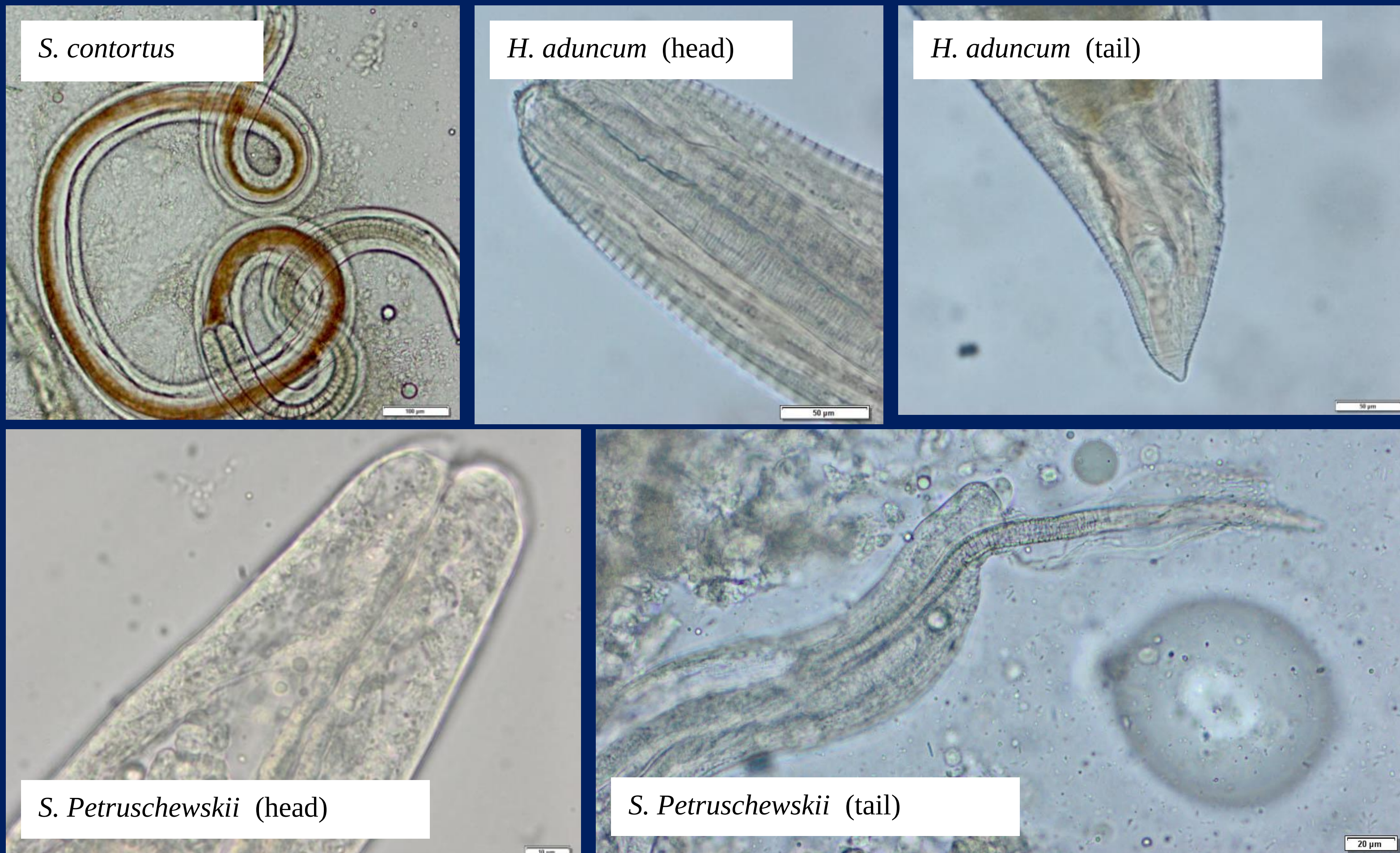

Figure 9. Identified nematoda parasites in toothcarp.

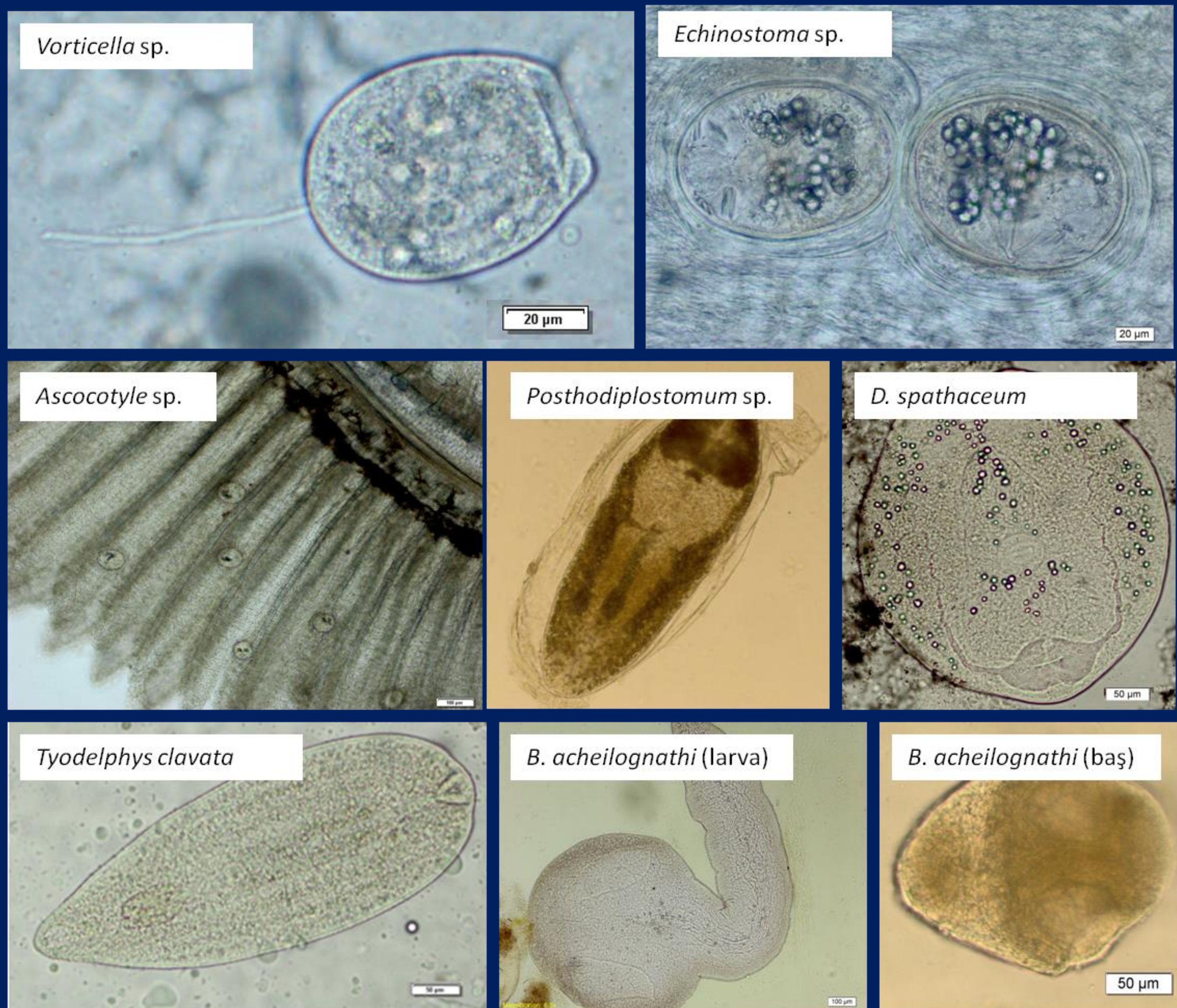

Figure 10. Identified protozoa, digenea and cestoda parasites in eastern mosquitofish.

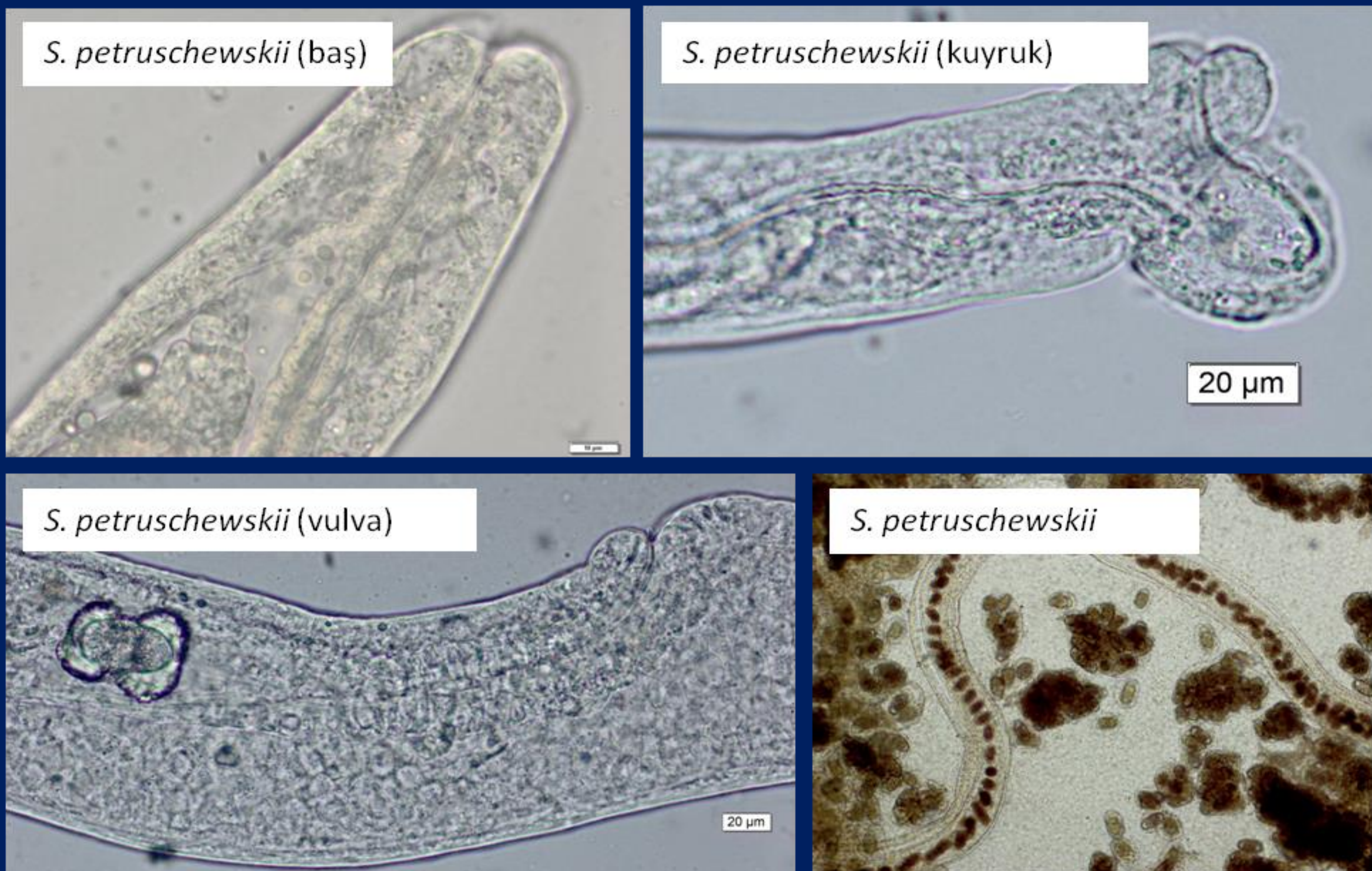

Figure 11. Identified nematoda parasite in eastern mosquitofish

The majority of trichodinids species are seldom involved in fish mortalities. However, Trichodinids are known to proliferate massively on stressed fish and become highly pathogenic under these circumstances.

Digenetic trematodes *Posthodiplostomum* sp., *Thylodelphys clavata* and *Diplostomum spathaceum* are also the members of Diplostomatidae and they do not have a strict host specificity,

Tylodelphys clavata and *Diplostomum spathaceum* are associated with specific effects or non-specific side effects of parasites, including impairment of vision that leads to exophthalmus, cataract and even complete collapse of the eye, which may be the cause of growth inhibition and death of significant portions of cultured fish,

Acanthocephalans are ‘thorny’ or ‘spiny headed’ worms with aquatic life cycles; fish as final or paratenic hosts and crustaceans as intermediate hosts,

Adults feed on the intestinal walls of vertebrates, especially freshwater and marine fishes. Intestinal sections showing massive epithelial sloughing and haemorrhages due to piercing of tissues with the hooks,

In heavily infected fish acanthocephalans may perforate the gut wall with their proboscis and *Neoechinorhynchus* sp. capable of causing irreversible damage to the intestinal tissues can seriously affect the health and quality of the *M. cephalus* brood stock and thereby making the fish unfit for hatchery production,

Table 3. Statistical test of equality of mean weight and length measurements (± standard error) of toothcarp with and without each particular parasite group when considered independently of the others present (P= probability value of acceptance)

Parasite group	Without=0 With =1	n	WEIGHT Mean ± SE	T-Test $H_0: m_0=m_1$	LENGTH Mean ± SE	T-Test $H_0: m_0=m_1$
PROTOZOA	0	59	1.386 ± 0.08	P =0.589	4.056 ± 0.06	P =0.583
	1	66	1.327 ± 0.07		4.003 ± 0.06	
MONOGENEA	0	90	1.306 ± 0.05	P* = 0.285	4.028 ± 0.05	P = 0.994
	1	35	1.483 ± 0.14		4.029 ± 0.09	
DIGENEA	0	1	0.6	-	3.5	-
	1	124	1.361 ± 0.05		4.032± 0.05	
NEMATODA	0	114	1.366 ± 0.05	P* = 0.625	4.091 ± 0.16	P* = 0.80
	1	11	1.245 ± 0.17		4.029 ± 0.09	
CESTODA	0	124	1.356 ± 0.05	-	4.028 ± 0.05	-
	1	1	1.2		4	
ACANTHOCEPHALA	0	120	1.355 ± 0.05	P* =0.989	4.030 ± 0.05	P* =0.59
	1	5	1.340 ± 0.25		3.980 ± 0.30	
COPEPODA	0	112	1.326 ± 0.06	P = 0.114	4.000± 0.05	P = 0.166
	1	13	1.608 ± 0.14		4.220 ± 0.11	

* Mann-Whitney test result

Table 4. Mean values (± standart error), variation range (Ax=first quartile- third quartile) and Student-t test of relative condition factor (K_n) of toothcarp infected and non-infected by each parasite group independently of the others present (P= probability value of acceptance)

Parasite group	Without=0 With =1	n	Kn ± SE	Ax	P-value for t-test H_0: Kn=1	Interpretation
PROTOZOA	0	59	1.017± 0.03	0.925 – 1.073	0.560	Kn = 1
	1	66	1.025 ± 0.02	0.845 – 1.134	0.302	Kn = 1
MONOGENEA	0	90	1.005 ± 0.02	0.885 – 1.197	0.820	Kn = 1
	1	35	1.065 ± 0.03	0.826 – 1.293	0.099	Kn = 1
DIGENEA	**0**	1	0.803	-	-	-
	1	124	1.024 ± 0.02	0.871 – 1.157	0.222	Kn = 1
NEMATODA	0	114	1.035 ± 0.02	0.892 – 1.178	0.077	Kn = 1
	1	11	0.881 ± 0.06	0.775 – 1.055	0.070	Kn= 1
CESTODA	0	124	1.022 ± 0.02	0.858 – 1.157	0.255	Kn = 1
	1	1	0.992	-	-	-
ACANTHOCEPHALA	**0**	120	1.019 ± 0.02	0.858 – 1.143	0.339	Kn = 1
	1	5	1.092 ± 0.08	0.943 – 1.210	0.318	Kn = 1
COPEPODA	**0**	112	1.068 ± 0.06	0.871 – 1.143	0.275	Kn = 1
	1	13	1.016 ± 0.02	0.838 – 1.263	0.418	Kn= 1

* - significant at 0.05 level

Table 5. Statistical test of equality of mean weight and length measurements (± standard error) of eastern mosquitofish with and without each particular parasite group when considered independently of the others present (P= probability value of acceptance)

			WEIGHT		LENGTH	
Parasite group	**Without=0 With =1**	**n**	**Mean ± SE**	**T-Test $H_0: m_0=m_1$**	**Mean ± SE**	**T-Test $H_0: m_0=m_1$**
PROTOZOA	0	156	0.627 ± 0.02	P =0.169	3.573 ± 0.06	
	1	19	0.716 ± 0.06		3.811 ± 0.04	P =0.583
MONOGENEA	0	174	0.636 ± 0.02	-	3.858 ± 0.12	
	1	1	0.7		3.814 ± 0.02	P* = 0.994
DIGENEA	0	17	0.641 ± 0.06	P =0.940	4.1	
	1	158	0.636 ± 0.02	P =0.169	3.818 ± 0.10	-
NEMATODA	0	152	0.626 ± 0.02		3.790 ± 0.04	
	1	23	0.682 ± 0.04	P = 0.373	3.987 ± 0.10	P* = 0.80
CESTODA	0	151	0.622 ± 0.02		3.798 ± 0.04	
	1	24	0.725 ± 0.04	P =0.079	3.929 ± 0.08	P =0.237

* Mann-Whitney test result

Table 6. Mean values (± standart error), variation range (Ax=first quartile- third quartile) and Student-t test of relative condition factor (K_n) of eastern mosquitofish infected and non-infected by each parasite group independently of the others present (P= probability value of acceptance)

Parasite group	Without=0 With =1	n	Kn ± SE	Ax	P-value for t-test H_0: Kn=1	Interpretation
Not parasitized		52	1.022±0.03	0.828 – 1.206	0.50	Kn = 1
PROTOZOA	0	156	1.025± 0.02	0.887 – 1.116	0.156	Kn = 1
	1	19	1.147 ± 0.07	0.992 – 1.231	0.051	Kn = 1
MONOGENEA	0	174	1.039 ± 0.02	0.888 – 1.126	0.031*	Kn > 1
	1	1	0.957	-	-	-
DIGENEA	0	17	1.039 ± 0.04	0.942 – 1.205	0.344	Kn = 1
	1	158	1.038 ± 0.01	0.887 – 1.119	0.048*	Kn > 1
NEMATODA	0	152	1.041 ± 0.02	0.884 – 1.128	0.024*	Kn > 1
	1	23	1.021 ± 0.06	0.885 – 1.062	0.747	Kn= 1
CESTODA	0	151	1.025 ± 0.02	0.885 – 1.116	0.174	Kn = 1
	1	24	1.122 ± 0.05	0.885 – 1.116	0.039*	Kn > 1

* - significant at 0.05 level

Table 7. Effect of co-infection of different parasite groups on the relative condition factor (Kn) of toothcarp

Parasites	n	Mean(Kn)	SE	Ax		P-value for t-test H_0: Kn=1	Interpretation
D	40	0.9918	0.037	0.871	1.079	0.826	Kn=1
D-M	7	1.180	0.085	1.027	1.315	0.077	Kn=1
D-P	32	1.035	0.033	0.908	1.203	0.306	Kn=1
D-Co	2	0.995	0.184	–	–	0.982	Kn=1
D-A	2	1.210	0.0002	–	–	0.001*	Kn>1
D-N	7	0.953	0.080	0.926	1.075	0.578	Kn=1
D-M-P	17	1.026	0.050	0.829	1.192	0.614	Kn=1
D-P-Co	3	0.961	0.072	0.850	1.096	0.643	Kn=1
D-P-N	1	0.785	–	–	–	–	–
D-P-A	1	1.158	–	–	–	–	–
D-M-Co	1	1.027	–	–	–	–	–
D-P-Ce	1	0.992	–	–	–	–	–
Co-M-P	1	0.804	–	–	–	–	–
D-Co-M-P	6	1.197	0.090	1.004	1.373	0.079	Kn=1
D-M-P-N	2	0.729	0.067	–	–	0.154	Kn=1
D-P-A-N	1	0.776	–	–	–	–	–
D-M-P-A	1	1.111	–	–	–	–	–

M= Monogenea, P= Protozoa, D= Digenea, A= Acanthocephala, Co= Copepoda, N=Nematoda, Ce=Cestoda; (* - significant at 0.05 level)

Table 8. Effect of co-infection of different parasite groups on the relative condition factor (Kn) of eastern mosquitofish.

Parasites	n	Mean(Kn)	SE	Ax		P-value for t-test H_0: Kn=1	Interpretation
D	114	1.012	0.018	0.881	1.119	0.520	Kn=1
P	6	1.022	0.068	0.865	1.193	0.758	Kn=1
N	7	1.104	0.070	0.975	1.296	0.190	Kn=1
Ce	3	1.003	0.024	0.957	1.037	0.905	Kn=1
D-N	13	0.992	0.111	0.809	0.986	0.943	Kn=1
D-Ce	18	1.122	0.057	0.968	1.255	0.047*	Kn>1
D-P	9	1.185	0.119	0.975	1.381	0.158	Kn=1
D-P-Ce	2	1.462	0.408	–	–	0.460	Kn=1
D-P-N	2	1.039	0.023	–	–	0.346	Kn=1
D-M-Co	1	0.957	–	–	–	_	_

M= Monogenea, P= Protozoa, D= Digenea, A= Acanthocephala, Co= Copepoda, N=Nematoda, Ce=Cestoda; (* - significant at 0.05 level)

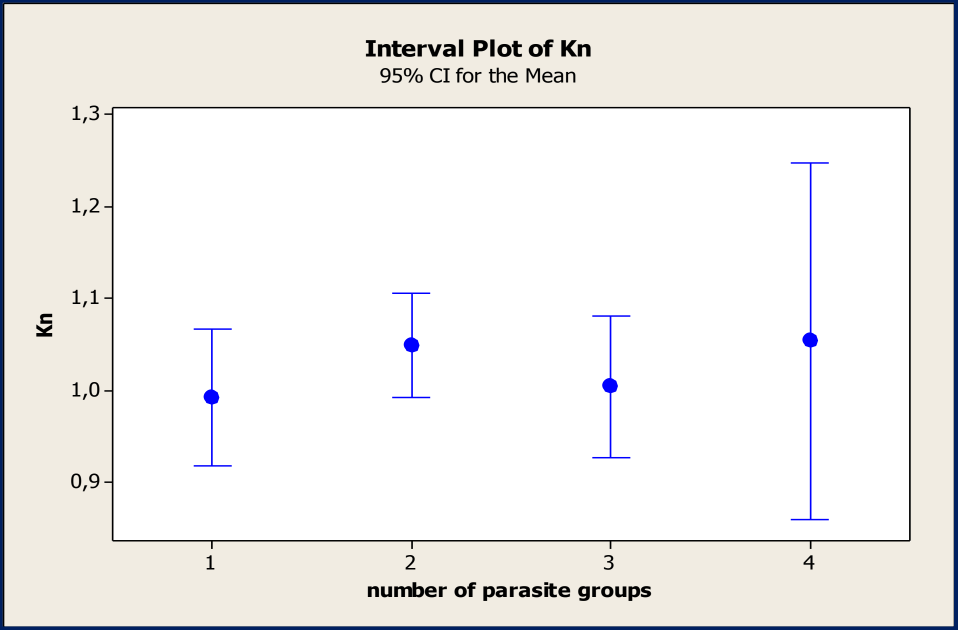

Fig 11. Influence of parasite load on the fish condition of the toothcarp

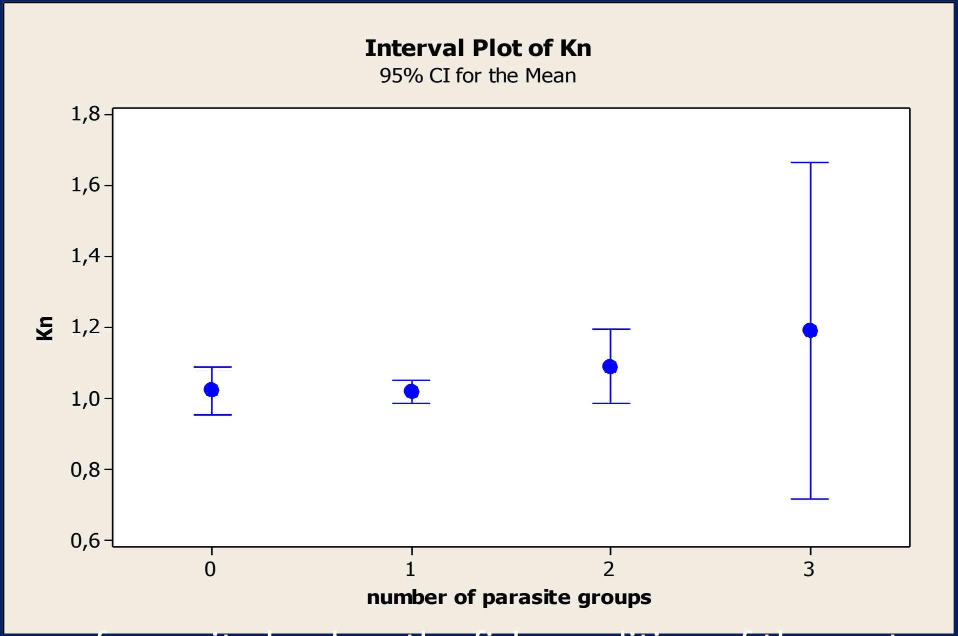

Fig 12. Influence of parasite load on the fish condition of the eastern mosquitofish

Table 9. Seasonal effect on the mean values of relative condition factor (Kn)

Season	Fish	n	Mean (Kn)	SE	P-value for t-test H_0: Kn=1	P-value for t-test H_0: Kn(A)=Kn(G)
Autumn	A	14	0.908[a]	0.04	0.076 Kn=1	0.015 Kn(A)<Kn(G)
	G	32	1.055[b]	0.02	0.039 Kn>1	
Spring	A	34	0.922[a]	0.03	0.016 Kn<1	0.013 Kn(A)<Kn(G)
	G	49	1.061[b]	0.04	0.137 Kn=1	
Summer	A	36	1.115[b]	0.03	0.001 Kn>1	0.009 Kn(A)>Kn(G)
	G	65	0.998[a]	0.03	0.962 Kn=1	
Winter	A	41	1.062[a]	0.03	0.096 Kn=1	0.873 Kn(A)=Kn(G)
	G	29	1.071[a]	0.04	0.152 Kn=1	
Overall	A	125	1.022[a]	0.019	0.609 Kn(A) = Kn(G)	
	G	227	1.035[a]	0.015		

Aphanius ANOVA P=0.000 (Autumn, Spring Kn<1)[a] ; (Summer, Winter Kn=1 or Kn>1)[b]
Gambusia ANOVA P=0.391 (Autumn, Spring, Summer, Winter)[a]

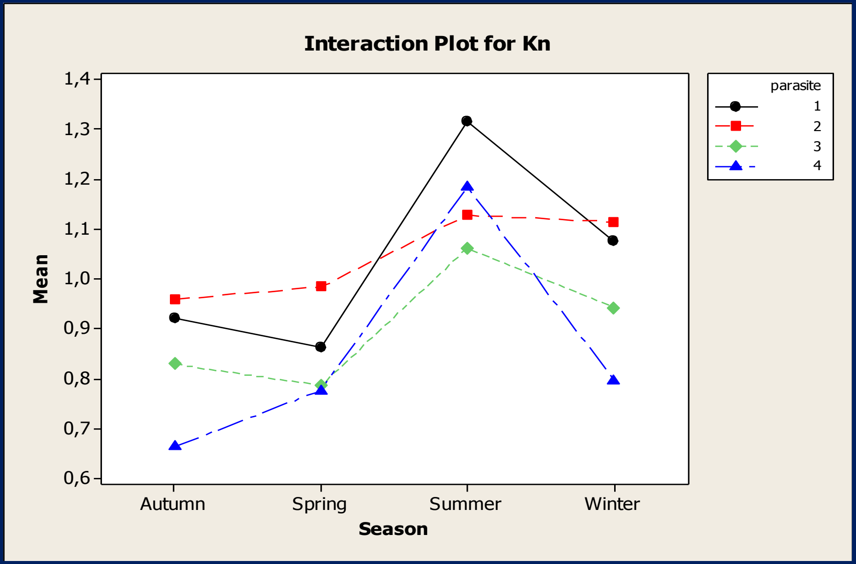

Fig 13. Influence of season on the fish condition of the toothcarp

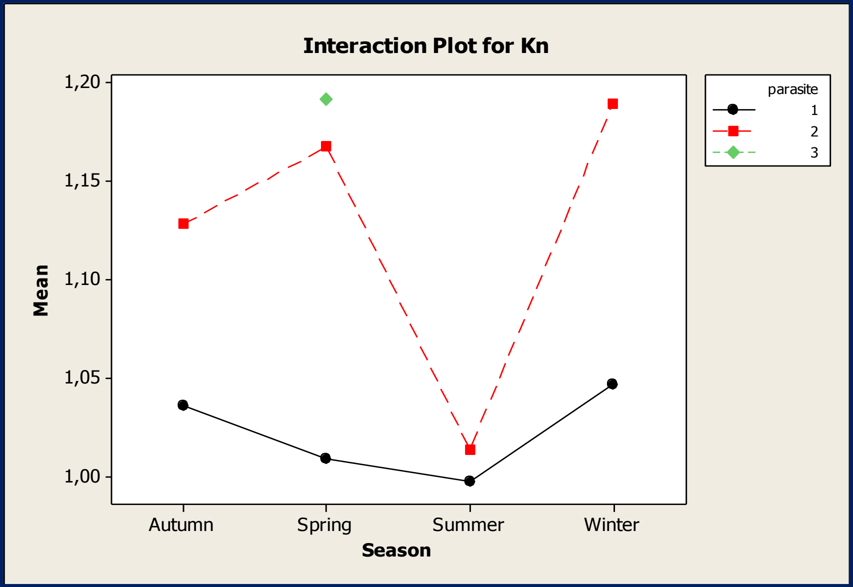

Fig 14. Influence of season on the fish condition of the eastern mosquitofish

4. Conclusions

- Amongst the observed parasite groups, ***Protozoa*** shows the highest abundance (Table 2), which is resulted from more space and food on higher weight and larger length of the fish if it is considered notwithstandingly the existence of the other parasite groups (Table 3),

- Amongst the observed parasite groups, ***Digenea*** shows the highest prevalence (Table 2), which is resulted from more food intake of the fish if it is considered notwithstandingly the existence of the other parasite groups (Table 3),

- Direct impact of ***Monogenean, Acanthocephalan, Copepoda*** and ***Cestoda*** infections seem to negligible due to very low level of infections, clearly co-infection with ***Protozoan*** and ***Nematode*** parasites along with above mentioned parasite groups (Table 7,8) did not cause any decreases in the development of both fish species,

- Moreover, co-infection with ***Protozoa*** and ***Digenea*** having the highest prevalence and mean intensity does not seem to cause damage to these fish, whose relative condition factor was equal to the expected value (Table 7,8) which corresponds to the needs of both parasite groups,

- Seasonal influences of co-infections by all parasite groups were determined in Autumn and Spring (Table 9) on the condition of toothcarp,

- Parasitic effect in number, varying from one to four, exhibited no adverse effect on the weight and in the mean condition factor as the number of group infections increases. The lack of a statistically significant negative impact of such parasitism may have been due to the small number of infected specimens of both fish species,

- Interaction of season and number of parasite groups appeared to be non-effective on the relative condition factor of both fish species.

- This study clearly revealed that endemic toothcarp has a well-balanced well-being despite 18 parasite species over its body,

- Similarly, introduced eastern mosquitofish also has a well-balanced well-being and this situation may have competitive advantage for this species over endemic toothcarp.

THANK YOU FOR YOUR ATTENTION

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