

Norileca indica (Crustacea: Isopoda, Cymothoidae) Infects *Rastrelliger kanagurta* Along the Malabar Coast of India – Seasonal Variation in the Prevalence and Aspects of Host-parasite Interactions

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This paper reports seasonal variations in the prevalence of host-parasite associations between *Norileca indica*, a cymothoid, and the Indian mackerel *Rastrelliger kanagurta* along the Malabar Coast of India. Eighty-eight marine fish species belonging to diverse families were examined, and only *R. kanagurta* was shown to be parasitized by *N. indica*, indicating a narrow host specificity. The prevalence, mean intensity, and abundance were 30.70, 1.71, and 0.52%, respectively. Different life cycle of this parasite, from larva to adult, infect the host fish. In most instances, a pair of *N. indica* infected the host, and in these instances a male-female combination was the most common (89.17%). The monthly occurrence of *N. indica* was charted for a period of 38 months (July 2012 to July 2014; March 2017 to March 2018), and statistical comparison of the data showed a significant difference ($p < 0.001$) among seasons. A positive correlation ($r = 0.40$) was observed between the size of female parasites and that of their respective host fish. There was a positive correlation ($r = 0.78$) between the size of female parasites and their fecundity. In all instances, adult *N. indica* individuals were found to specifically attach to the mucus membrane of branchial operculum, causing visible physical damage, including atrophy (reduced length) of the gill filaments and overall loss of gill normalcy. Furthermore, permanent occupancy by female *N. indica* resulted in the formation of a deep pit in the gill chamber floor and also caused atrophy of gill filaments. Overall, our findings yielded a greater understanding of the occurrence, season-wise prevalence, and potential host-parasite associations of *N. indica*.

Key words: Parasitic cymothoid, Prevalence, Seasonal variation, Host-parasite association, India.

BACKGROUND

Cymothoids are obligate ectoparasitic isopods comprising 40 recognized genera and more than 380 species, which infect a wide range of marine and freshwater fishes worldwide (Ahyong et al. 2011; Trilles

1969 1994; Smit et al. 2014 in review; Aneesh et al. 2015, 2018; Panakkool-Thamban et al. 2016). They have attracted the attention of researchers worldwide as they have deleterious impacts on the health of host fishes and thereby seriously limit aquaculture productivity and economic viability (Adlard and Lester 1994; Bunkley-

Williams and Williams 1998, Fogelman et al. 2009; Smit et al. 2014 in review). Cymothoids partially invade the host tissue, resulting in serious physical and physiological damage such as reduced growth, impaired reproduction, behavioral alterations, and, in extreme cases, death (Smit et al. 2014). However, little or nothing is known about the cymothoid fauna in some parts of the world. The literature suggests that knowledge on the occurrence of cymothoids parasitizing Indian marine fishes is still scant; until now, 46 species belonging to the family Cymothoidae have been recorded from Indian fishes (Rameshkumar et al. 2013a).

The cymothoid genus *Norileca* (Milne Edwards 1840) comprises three reported species: *N. borealis* (Javed and Yasmeen 1999), *N. triangulata* (Richardson 1910), and *N. indica* (Milne Edwards 1840), all of which infect the branchial cavity of pelagic marine fishes worldwide (Remeshkumar and Ravichandran 2015; Serita van der Wall et al. 2017). *Norileca indica*, the most common species, has been reported from different geographical regions, including the Zambezi estuary; Mozambique and Tanjona Vilanandro; the North-Western coast of Madagascar (Rokicki 1982; Serita van der Wal et al. 2017); Mayotte Island (Trilles 1976); Pakistan (Behera et al. 2016); India (Rameshkumar et al. 2013b 2015; Dipanjan et al. 2016); the Indian Eastern coast (Behera et al. 2016); Thailand (Nagasawa and Petchsupa 2009); Indonesia (Milne Edwards 1840; Schioedte and Meinert 1884; Trilles 1979); China (Yu and Li 2003); Mariveles and Luzon Islands (Schioedte and Meinert 1884; Trilles 1976; Yamauchi et al. 2005); Panay Gulf, Province of Iloilo, the Philippines (Cruz-Lacierda and Nagasawa 2017); the Arafura Sea, Northern Territory coast of Australia (Bruce 1990); and North-Western Australia (Avdeev 1978). However, no specific study has been reported on the occurrence of *N. indica* along the South-West Indian coast, which make up a large part of the fishing industry in India (Madhupratap et al. 2001). The present paper primarily reports on the parasitic occurrence of *N. indica* in the Malabar Coast of India on a monthly and seasonal basis. This study also focuses on the host-parasite interaction in terms of host specificity, site specificity, microhabitat preference, correlation of host and parasite size, and parasite size and fecundity. Additionally, we analyzed the damage caused to the fish host.

MATERIALS AND METHODS

Fishes were collected from the following major fish landing centers on the Malabar Coast: Ayyikkara (11°51'N, 75°22'E), Azhikkal (11°56'0"N 75°18'0"E),

and Chombala (11°39'N 75°33'E) during the period from July 2012 to July 2014 and March 2017 to March 2018. Freshly caught fishes were collected directly from the fishing boats of local fishermen. Samples were taken two or three times per week throughout the study period. At the sampling location itself, fish body parts (body surface, lateral line region, base of the pectoral fin, branchial cavity and gill filaments, inner wall of the operculum, etc.) were closely examined for parasitic isopods using a hand lens. The infection site was documented. The total length of each host fish was measured using a metric ruler (in centimeters, cm). From each of the juvenile, male, transitional, and female stages of the parasites, 12 parasites were randomly sampled and their lengths measured in millimeters (mm). The host fishes and parasites were photographed by an Olympus camera (μ TOUGH-3000) with the stereo microscope Leica-S6D. Recovered parasites were preserved in 70% ethanol (Ramakrishna 1980) for taxonomic identification and documentation. The identification was performed using a dissection microscope and a stereo microscope Leica-S6D, according to Trilles (1968 1972). The identification of life cycle stages and classification of female stages of *N. indica* were done according to Bakenhaster et al. (2006), Panakkool-Thamban et al. (2016), and Helna (2016). Prevalence (P), intensity (I), and abundance (A) of parasitic infection were calculated according to Margolis et al. (1982) and Bush et al. (1997) using the software Quantitative Parasitology 3.0 (Reiczigel and Rózsa 2005). Seasonal prevalence was analyzed by one-way ANOVA and paired *t*-test; $p < 0.001$ was considered highly significant (**) and $p < 0.05$ was considered significant (*). A Bonferroni test was applied to correct the *p* value. Eggs from the brood pouch were carefully removed and fecundity was calculated by counting the number of eggs. Host nomenclature and fish taxonomy were confirmed according to Fish Base (Froese and Pauly 2018). Voucher specimens of *N. indica* (ISO-NI-F1) were deposited in the Parasitic Crustacean Museum, Crustacean Biology Research Laboratory, Sree Narayana College, Kannur, Kerala, India.

RESULTS

Occurrence of *Norileca indica*

Out of the 3752 individuals of *Rastrelliger kanagurta* surveyed, 1152 showed infection with the cymothoid *N. indica*; the prevalence, intensity, and abundance were recorded as 30.70%, 1.71, and 0.52, respectively. A total of 1962 parasite specimens were

recovered at different life cycle stages: 1062 females (54.12%), 810 males (41.28%), 76 transitionals (3.87%), and 10 juveniles (0.50%) (Figs. 1 and 2; Table 1). In two instances, a pair of infective stage manca II was also recovered (Fig. 2C). A boxplot representation of the size (length) variation in recovered parasites at different life cycle stages is given in figure 3. There was a steep increase in the length of parasites in the sequential developmental stages from juvenile to male, transitional, and female stages.

In most instances (804/1962 recovered parasites), two *N. indica* individuals simultaneously parasitized

one host. Among them, 89.17% (717/804) were male-female, 7.46% (60/804) were male-transitional, 0.37% (3/804) were female-juvenile, 0.62% (5/804) were juvenile-male, 0.12% (1/804) were male-male, 0.49% (4/804) were female-transitional, 0.12% (1/804) were juvenile-juvenile, and 1.61% (13/804) were female-female (Fig. 4). Of the remaining fish individuals with a single parasite (17.83%; 350/1962), 312 were female (89.14%), 26 were male (7.42%), and 12 were transitional (3.42%) (Table 1). Of these fish occupied by a single parasite, the vacant gill chamber showed a deep pit-like depression, indicating that a parasite had been

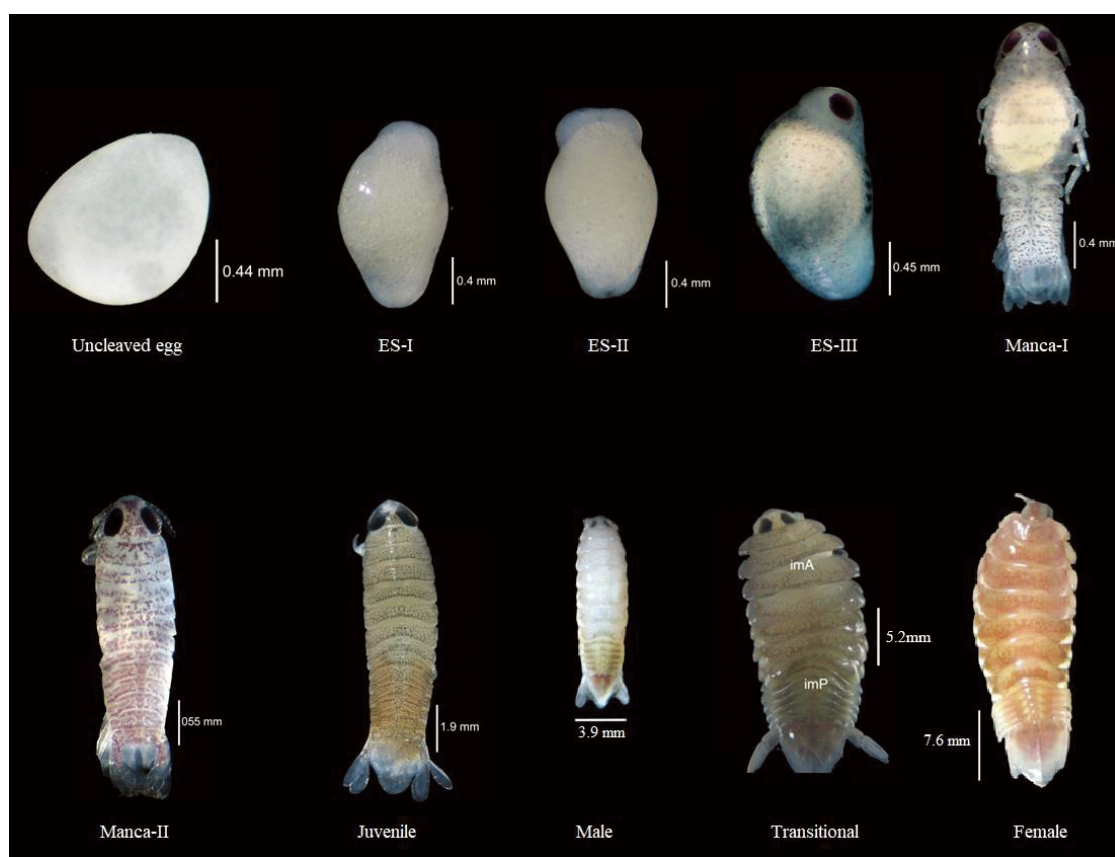


Fig. 1. Life cycle stages of *N. indica* recovered from *R. kanagurta*. ES-I, ES-II, and ES-III: Eggs undergoing embryonic development; Manca-I and Manca-II: larval stages; male, transitional, and female: adult stages.

Table 1. Parasitological index of *Norileca indica*

Parasite and host fish	NFO	NFI	P%	NPR					I	A
				F	M	T	J	ML		
<i>Norileca indica</i>	3752	1152	30.70	1062	810	76	10	4	1.70	0.52
<i>Rastrelliger kanagurta</i>										
Unpaired parasites (single occupancy)	-	-	-	312	26	12	-	-	-	-

NFO: number of fishes observed, NFI: number of fishes infested, P-prevalence, NPR-number of parasites recovered, F-female, M-male, I-intensity, A-Abundance, T- transitional, J-juvenile, ML- manca larva. Total number of parasite recovered (NPR) = 1962.

there before. In total, 1062 females were recovered, 903 of which were further classified according to the condition of their brood pouch. 40% of females carried eggs (uncleaved or embryonic stage), 23% carried larvae in their marsupium, 19% had an empty brood pouch, and 18% had no brood pouch (Fig. 5).

Monthly and seasonal variations in prevalence

The prevalence of *N. indica* in *R. kanagurta* exhibited monthly variation ranging from 11.6 to 51.6%; the maximum prevalence was recorded in March and minimum prevalence in September. From October,



Fig. 2. Infection by *N. indica* – site of attachment, tissue damage, and adaptations for clinging to the host fish. A, Male-Female (♀ - ♂) pair in the gill chamber (branchial cavity) of *R. kanagurta*; B, juvenile- ♂ pair; C, Manca-II (first infective stage); D and E, damaged gill chamber (arrow) and gills due to the infection of *N. indica*; F, gill of uninfected fish j- Juvenile, m-manca-I. G, male pereopods; H, female pereopods; I, mouthpart complex.

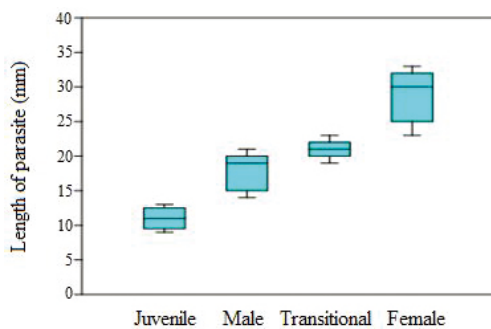


Fig. 3. Box plot of *N. indica* size (length) at different infective lifecycle stages (Juvenile, Male, Transition, Female) recovered from the host fish, *R. kanagurta*.

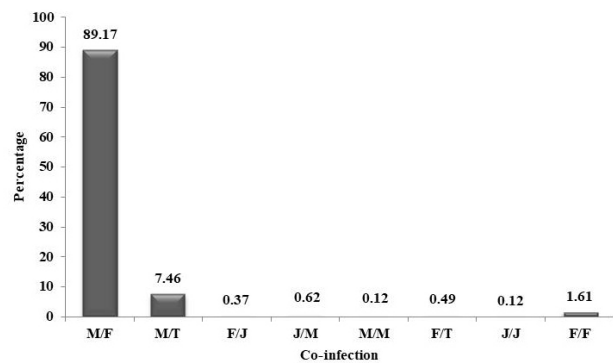


Fig. 4. Occurrence of *N. indica* in pairs with different life cycle stage combinations. M/F: male-female (89.17%; 717/804), male-transitional (7.46%; 60/804), female-juvenile (0.37%; 3/804), juvenile-male (0.62%; 5/804), male-male (0.12%; 1/804), female-transitional (0.49%; 4/804), juvenile-juvenile (0.12%; 1/804), and female-female (1.61%; 13/804).

the prevalence of *N. indica* showed a steady increase from 19.66% and maximum prevalence was noticed in March (51.66%). During April and May, the prevalence of *N. indica* infection was high and stable (46.66% and 47%, respectively); prevalence then dropped over the subsequent months until September (Fig. 6). In order to analyze the variation in prevalence across seasons, the months were categorized into winter (October-January), pre-summer/summer (February-May), and monsoon/post-monsoon months (June-September). *Norileca indica* presence fluctuated significantly across seasons (Fig. 7). The prevalence was significantly highest (p

$= 0.001$) during the pre-summer/summer months, and declined significantly ($p = 0.029$) during the monsoon/post-monsoon periods. However, the prevalence was not statistically significant ($p = 0.098$) between winter and monsoon/post-monsoon periods. According to the Bonferroni test, the rate of prevalence was significantly different between the following periods; pre-summer/summer and winter, monsoon/post-monsoon and pre-summer/summer. There was no significant difference in the rate of prevalence between monsoon/post-monsoon and winter.

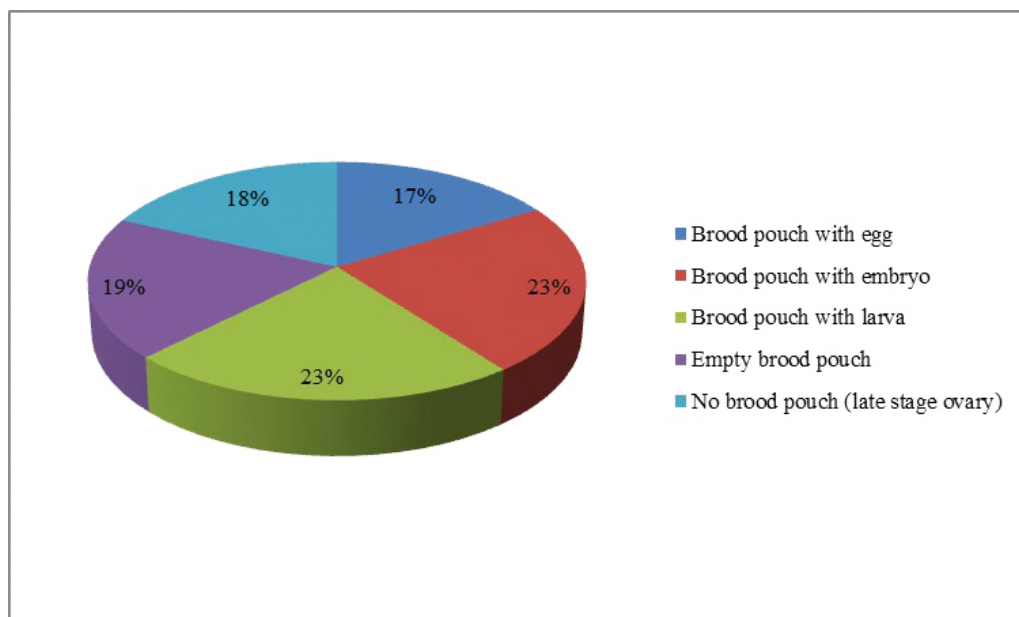


Fig. 5. *Norileca indica*—proportion of female stages recovered.

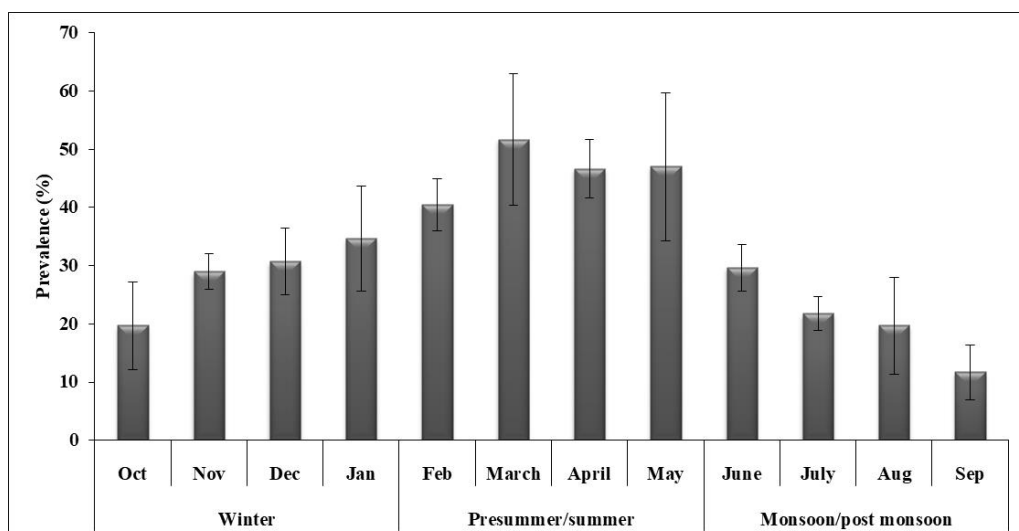


Fig. 6. Monthly prevalence of *N. indica* along the Malabar Coast of Kerala during the study period.

Correlations between host and parasite

Of the 88 marine fish species in the families surveyed (Scombridae, Stromateidae, Carangidae, Teraponidae, Ambassidae, Engraulidae, Belonidae, Clupeidae, Dasyatidae, etc.), the cymothoid *N. indica* was recovered only from the Indian mackerel *Rastrelliger kanagurta*, suggesting that this is its only host in the Malabar Coast. In all instances, the brachial chamber of the host fish appeared to be the only site that *N. indica* infected (Fig. 2). The parasite was found

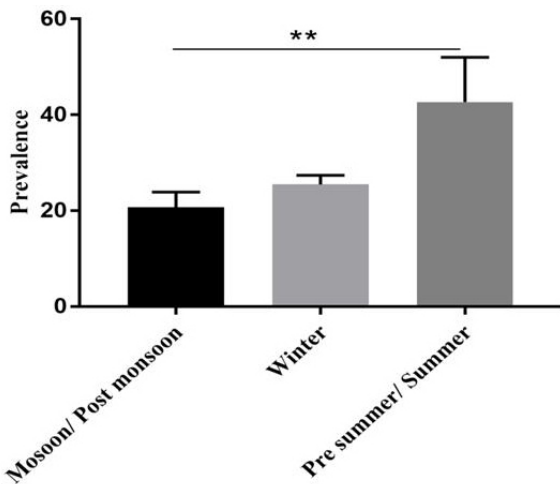


Fig. 7. Seasonal variation in the prevalence of *N. indica* along the Malabar Coast of Kerala. Overall variations among the three seasons was statistically shown using one-way ANOVA (** $p = 0.0011$). Paired *t*-test of prevalence between monsoon/post-monsoon and winter seasons (ns-no significance; $p = 0.0983$) and paired *t*-test of prevalence at pre-summer/summer vs monsoon/post-monsoon were significantly different ($p = 0.0293$).

settled in the chamber with its dorsal side facing the brachial floor and abdomen facing the operculum. It used the pereopods to cling to the mucus membrane of the operculum; the cephalon was oriented towards the anterior end of the host fish (Fig. 2A). The shape of the female body was shown to have characteristic asymmetry depending on whether it occupied the right or left branchial chamber of the host—the body of those occupying the right brachial cavity was hunched to the left and that occupying the left cavity was hunched to the right.

Norileca indica was found to be significantly more prevalent in large host fish (total length: 16.1–24 cm) than small ones (total length: 12–16 cm) ($p = 0.048$). Larger fish harbored larger parasites, and most small fish harbored juvenile parasites. A positive correlation was observed between host fish and parasite size ($R^2 = 0.16$, $r = 0.40$; $N = 46$) (Fig. 8).

The fecundity of *N. indica* ranged from 64–540, and parasite size ranged from 23.5–34.0 mm. A total of 18 female parasites were sampled for fecundity analysis. A positive correlation was observed between the size of female *N. indica* and their fecundity ($R^2 = 0.61$, $r = 0.78$; $N = 18$) (Fig. 8).

The continuous lodging of parasites resulted in the formation of a large pit in the gill chamber. The physical forces exerted by the parasite on the gills might be the reason why the gill filaments often atrophied, especially those of the 1st and 2nd holobranchs. Its severity was found to be relatively high in gill chambers where large-sized female *N. indica* settled (Fig. 2D). The mucus membrane of the operculum also showed severe damage with copious secretions of mucous, likely due to the clinging of the parasite.

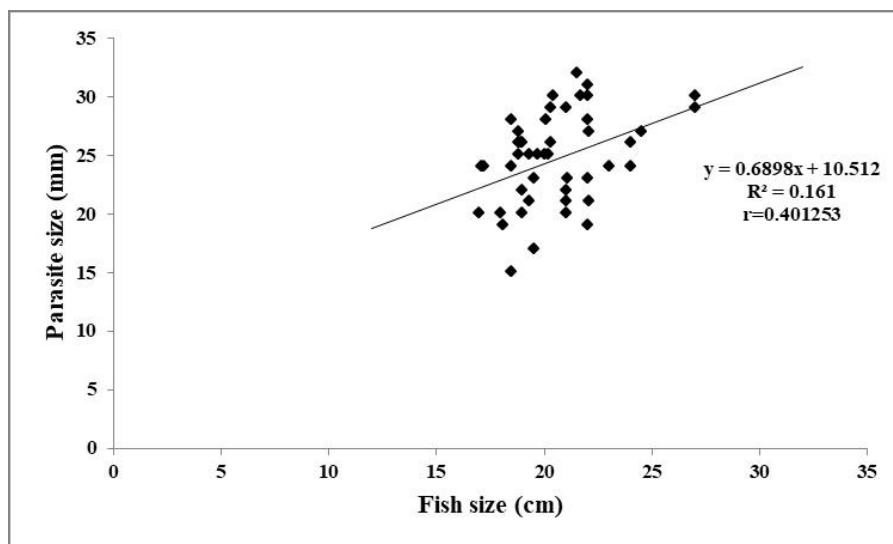


Fig. 8. Correlation between fish size (*R. kanagurta*) and parasite size (*N. indica*).

DISCUSSION

The genus *Norileca* was established by Bruce (1990) for *Norileca indica* (Milne-Edwards 1840), previously reported as *Livoneca* (or *Lironeca*) *indica* or *Livoneca ornata* (Heller 1868) (Serita van der Wall et al. 2017). Nine species of marine fishes have thus far been reported to host *N. indica*: five carangids, viz. *Alepes apercna* (Trilles 1976), *Atule mate* (Avdeev 1978), *Selar crumenophthalmus* (Rockicki 1982; Bruce 1990; Nagasawa and Petchupa 2009; Cruz-Lacierda and Nagasawa 2017; Serita van der Wall et al. 2017), *Decapterus russelli* (Ghani 2003), and *Carangoides malabaricus* (Kumar et al. 2017); the clupeid fish *Herklotichthyes* sp. (Bruce 1991; Ghani 2003); the Nemipterid fish *Nemipterus randalli*; the Leognathid fish *Secutor insidiator* (Behra et al. 2016); and the scombrid fish *Rastrelliger kanagurta* (Ghani 2003; Rameshkumar et al. 2014; Dipanjan et al. 2016).

Host specificity is one of the most important factors in host-parasite interactions, and it reflects host ecology, chorology, and phylogeny (Cressey et al. 1983). All three valid species of *Norileca* [*N. borealis* (Javed and Yasmeen 1999), *N. indica* and *N. triangulata* (Richardson 1910)] have been reported from at least one scombrid fish, indicating the affinity of the genus *Norileca* to infect scombrid fishes. During the present study, though 88 species of fishes were closely observed along the Malabar Coast, *N. indica* was recovered only from the scombrid *R. kanagurta* with high prevalence (30.70%), suggesting that this is its only host in the region. Other previously reported hosts of *N. indica* (i.e., *S. crumenophthalmus*, *S. insidiator*, *C. malabaricus*, and *D. russelli*) (Rameshkumar et al. 2013b; Neeraja et al. 2014; Serita van der Wall et al. 2017) showed no sign of infection in the Malabar Coast, signifying the influence of geographical environment in the host selection of cymothoids. For instance, *N. indica* exhibited different host specificities along two neighboring coastal areas, the Malabar Coast (present study) and Mumbai Coast (Neeraja et al. 2014) of the Indian subcontinent; only the carangid *S. crumenophthalmus* was reported to be a host in the Mumbai Coast. A previous study suggests that host specificity increases with decreasing latitude (Smit et al. 2014). This should be addressed in the case of *N. indica*, which prefer diverse host fish species from different geographical regions including Madagascar—five carangids, one scombrid, one nemipterid, one leognathid, and one clupeid (Rockicki 1982; Serita van der Wall et al. 2017)—Thailand (Nagasawa and Petchupa 2009), Indonesia (Milne Edwards 1840; Trilles 1979), China (Yu and Li 2003), the Philippines (Cruz-Lacierda and Nagasawa 2017), Australia (Bruce 1990), and India (Rameshkumar et al. 2013 2015;

Dipanjan et al. 2016).

Norileca-host interactions are noteworthy as the parasite has a preference for attaching to the branchial cavity of the host fish during its parasitic mode of life (Bruce 1990; Javed and Yasmeen 1999; Ghani 2003; Panakkool-Thamban et al. 2016). The present study also supports the previous reports on the site-specific infections by *N. indica* (Bruce 1990; Neeraja et al. 2014; Rameshkumar et al. 2015; Behera et al. 2016, Serita van der Wall et al. 2017). However, *N. indica* has also been recovered from the buccal cavity of *D. russelli* from coastal waters of Indonesia (Pattipeiluhu and Gill 1998). Site specificity is determined by the needs of the parasite and the limitations exerted by host morphology and habits (Morton 1974). While parasitic crustaceans form increasingly intimate associations with their hosts, they presumably undergo considerable morphological and structural changes related to their attachment site and feeding habits (Saarinen and Taskinen 2005). *Norileca indica* is also adapted with hook-like pereopods to cling to the host tissue; the mouth part complex consists of paired maxillule, maxilla, maxilliped, and mandibles that are modified for blood feeding (Fig. 2G–I). The oesophageal side gland in *N. indica* also seems to facilitate blood sucking, as is evident from the basophilic and secretory nature of its cells, likely the anticoagulant. The body of ovigerous female members of *N. indica* are right or left hunched, depending on whether they are located in right or left branchial cavity. Since most of the reported cymothoids/parasitic isopods infecting the branchial region of the host fishes have this kind of left/right asymmetry, it is apparently an adaptation for the parasite to permanently occupancy the brachial chamber of the host fish.

After oviposition, the eggs and subsequently formed larvae are maintained in the brood pouch of the female until it is released into the water as a competent settling larvae (Manca-II), presumably an adaptive strategy to settle immediately on the host for its parasitic life. Yet, the relatively low number of mating groups (usually no more a pair according to our present data) suggests that settling of larvae is either rare or chemically controlled so no more than a pair settle next to each other (of the opposite sex).

Once the parasite settles in the host, through a series of molting it becomes juvenile, then male, transitional, and female (Fig. 9). In the case of *N. indica* (804/1962 recovered parasites), simultaneous infection by two individual parasites was observed. Among them, 89.17% were male-female, 7.46% male-transitional, 0.37% female-juvenile, 0.62% juvenile-male, 0.12% male-male, 0.49% female-transitional, 0.12% juvenile-juvenile, and 1.61% female-female. From these observations, it is evident that two individuals of

manca II can infect the fish simultaneously and become juvenile-juvenile, then male-male. Since one of the males becomes female and the other remains male, in double infections, the male-female combination was most prevalent (89.17%), indicating the possibility of environmental rather than genetic sex determination. But in single infections, the prevalence is the highest for females. Given that this species is protandrous hermaphroditic (Kottarathil and Kappalli 2019), this type of prevalence in the hosts as a function of mate availability might be a selective advantage.

Seasonality has also been reported in other marine parasites, including *PhrEXOcephalus umbellatus* (Ohtsuka et al. 2009) and *Lernaea cyprinacea* (Marcogliese 1991). In the Malabar Coast of India, different species of *Nerocila* show significant seasonal fluctuations (Aneesh et al. 2013). A recent study from our laboratory could also demonstrate a seasonal variation in the prevalence of the isopods *Joryma brachysoma*, *Anilocra leptosome*, and *Agarna malayi* and a copepod *Pseudorbitacolax varunae* infesting clupeidean fishes of the Malabar Coast; all of these are more prevalent during summer months and least during monsoon months (Rijin et al. 2019). The present study observed a more or less similar pattern of fluctuation in the infection rate of *N. indica* on *R. kanagurta*. The prevalence was significantly highest during the pre-summer/summer (February to May), reaching a maximum in March (51.66%). By the onset of the

monsoon season (June to September), the prevalence showed a downward trend, reaching the lowest value in September (11.66 ± 4.7). From there on, it showed a gradual increase over the winter months (October to November), peaking at 34.66 ± 8.98 in January.

The cyclic pattern of change in prevalence with the seasons in *N. indica* indicates that seasonal environmental parameters such as rainfall, salinity, and temperature apparently have a role in its obligatory ectoparasitic life in the host fish *R. kanagurta*. The low level of the prevalence during the monsoon and the post-monsoon months is likely induced by low salinity due to heavy rainfall, and the gradual increase in salinity until the end of the post-monsoon and winter months (October to January) seems to facilitate its gradual parasitic infection. Salinity usually peaks in the summer (February–May), which might be favorable for the parasite to infect its host fish, and the data appear to be akin to our research reports on isopods (Aneesh et al. 2013; Rijin et al. 2019). However, a detailed and systematic experimental study pertaining to this aspect—*i.e.*, whether salinity favors the isopod parasitization of fishes—would confirm this.

All female *N. indica* collected during the present study were reproductively active, as is evident from the presence of a brood pouch carrying the eggs or larvae undergoing different developmental stages. Salinity is known to induce differences in developmental capabilities of eggs and free swimming stages (De

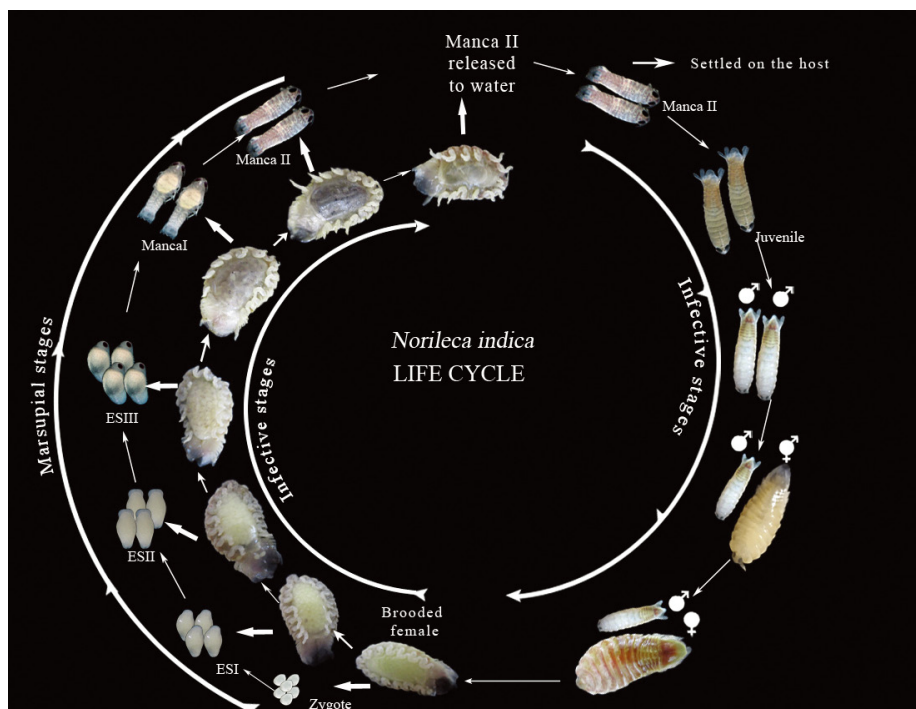


Fig. 9. Life cycle of *N. indica*, indicating the infective and marsupial stages.

Meeûs et al. 1992 1993). Many free-living crustaceans have been reported to require an optimum salinity larval development, and for them the monsoon season is not generally a breeding season (Sudha and Anilkumar 1996; Syama et al. 2010; Sudha et al. 2012). Previous reports also showed that reproduction in several cymothoids is influenced by environmental factors (Trilles 1969; Leonardos and Trilles 2003). High ambient water temperatures might be advantageous for some cymothoids to live and proliferate (Papapanagioutou and Trilles 2001; Aneesh et al. 2013; Rajkumar et al. 2005; Rijin et al. 2019). But the situation is different for *Nerocila serra*—which infests the sea snake *Enhydrina schistose*—and the pennilid copepod *Lernaenicus polynemi*, both of which are most prevalent during the monsoon season (Saravanakumar et al. 2012; Bharadhirajan et al. 2013). In addition, the copepods *Clavellisa hilsae*, *Peniculus fistula fistula*, and *Naobranchia cygniformis* infest clupeidean fishes, and are most prevalent after the monsoon season (Rijin et al. 2019). The prevalence shown by the cymothoids *Mothocya renardi* and *M. collettei* is more or less uniform at approximately 83% throughout the year (Panakkool-Thamban et al. 2016).

There also exists a positive correlation between fish size (*R. kanagurta*) and the size of the female parasite *N. indica*; in all instances, the juvenile (pre-adults) parasites were only recovered from the small sized (12–16 cm) host fish. These observations support previous reports in the parasitic isopods *Glossobius hemiramphi* (Bakenhaster 2006), *Anilocra pomacentri* (Fogelman and Grutter 2008), and *Mothocya renardi* (Panakkool-Thamban et al. 2016), all of which show a concomitant increase with the increase in their host fish size. There is a positive relationship between parasite

size and host size because large bodies are correlated with high fecundity, and therefore a large body size is selected for when larger hosts are present (Moranda and Sorcib 1998). This was supported by the positive correlation between female size and fecundity of *N. indica* in the present study ($R^2 = 0.61$, $r = 0.78$; Fig. 10).

The negative impacts of cymothoids depend on the parasite species and its location on the host (Trilles 1994). As a branchial parasite, the cymothoid *N. indica* also causes considerable damage to the branchial cavity and gills of the host fish *R. kanagurta*. Continuous lodging of the parasite results in the formation of deep pits on the floor of the gill chamber and atrophied gill filaments (Fig. 2D and E), akin to a recent report in other cymothoids (Panakkool-Thamban et al. 2016). In *N. indica*, the damage is more severe with larger parasites. The mucous production by the host fish and eroded or scratched mucous membrane of the host resulting from the parasite clinging likely leads to secondary infections, as reported by Fogelman et al. (2009). Previous reports also show that branchial cymothoids can form a pericardial cavity and result in heart decompression and reduced respiratory metabolism (Trilles 1994), whereas the buccal species degenerate the tongue (Romestand and Trilles 1979; Brusca and Gilligan 1983) and cause skull deformations (Trilles 1994) and teeth problems (Romestand and Trilles 1979). Isopods that affect the body surface also damage the host in multiple ways, such as degenerating the fins, damaging the scales and epidermis, and reducing the sustained aerobic swimming speed and endurance of parasitized fish (Ostlund-Nilsson et al. 2005). Impaired reproduction and a reduced lifespan have also been reported in some hosts (Adlard and Lester 1994).

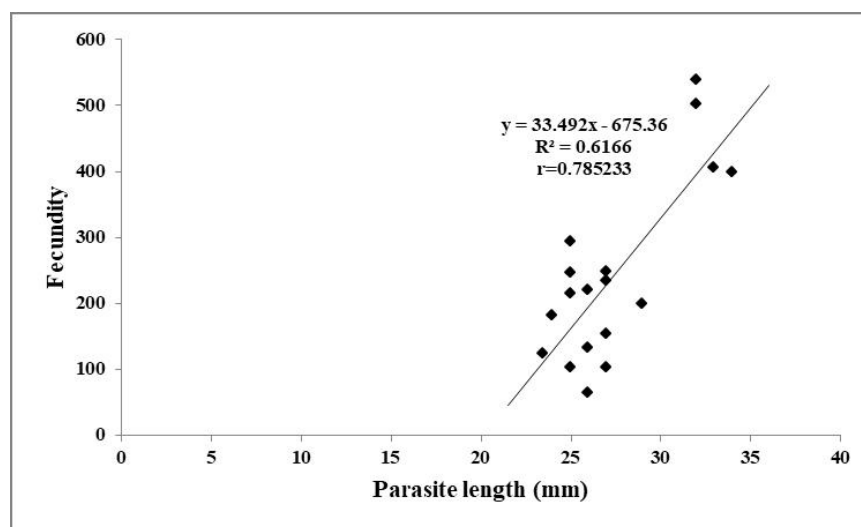


Fig. 10. Correlation between size of female parasites (*N. indica*) and their fecundity.

CONCLUSIONS

The present study shows that *Norileca indica*, an obligatory cymothoid parasite that infests *Rastrelliger kanagurata*, exhibits seasonal dependent variation in its prevalence, and that pre-summer/summer is the most favorable season for infection. There were regional differences in host preference, as well as a significant positive correlation between both host size and parasite size and parasite size and fecundity. This is significant in the context of host-parasite interactions in general. Exhibiting discrete host specificity and site specificity for their obligatory parasitism, as well as protandrous hermaphroditism, this profusely breeding cymothoid is an excellent model species for marine parasite research. Further studies on this cymothoid using modern biological approaches would unravel the intricacies of host-parasite interactions and the molecular mechanism of protandrous hermaphroditism, which in turn would be helpful for formulating essential strategies to effectively control cymothoid parasitism in aquaculture sectors.

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REFERENCES

- Adlard RD, Lester RJ. 1994. Dynamics of the interaction between the parasitic isopod, *Anilocra pomacentri*, and the coral reef fish, *Chromis nitida*. *Parasitology* **109**(3):311–324.
- Ahyong ST, Lowry JK, Alonso M, Bamber RN, Boxshall GA, Castro P, Gerken S, Karaman GS, Goy JW, Jones DS, Meland K, Rogers DC, Svavarsson J. 2011. Subphylum Crustacea Brünlich, 1772 Z.-Q. In: Zhang (Ed.), *Animal Biodiversity: An Outline of Higher-Level Classification and Survey of Taxonomic Richness*. *Zootaxa* **3148**:165–191. doi:10.11646/zootaxa.3148.1.33.
- Aneesh PT, Sudha K, Arshad K, Anilkumar G, Trilles JP. 2013. Seasonal fluctuation of the prevalence of cymothoids representing the genus *Nerocila* (Crustacea, Isopoda), parasitising commercially exploited marine fishes from the Malabar Coast, India. *Acta parasitologica* **58**(1):80–90.
- Aneesh PT, Sudha K, Helna AK, Anilkumar G. 2018. *Agarna Malayi* Tiwari 1952 (Crustacea: Isopoda: Cymothoidae) parasitising the marine fish, *Tenualosa toil* (Clupeidae) from India: re-description/description of parasite life cycle and patterns of occurrence. *Zool Stud* **57**:25 doi:10.6620/ZS.2018.57-25.
- Aneesh PT, Sudha K, Helna AK, Anilkumar G, Trilles JP. 2015. *Cymothoa frontalis*, a cymothoid isopod parasitizing the belonid fish *Strongylura strongylura* from the Malabar Coast (Kerala, India): redescription, description, prevalence and life cycle. *Zool Stud* **54**:42. doi:10.1186/s40555-015-0118-7.
- Avdeev VV. 1978. Parasitic isopods of the family Cymothoidae (Crustacea, Flabellifera) from the Red Sea. *Biologiya Morya* **4**:30–35. (in Russian)
- Bakenhaster MD, McBride RS, Price WW. 2006. Life history of *Glossobius hemiramphi* (Isopods: Cymothoidae): Development, Reproduction, and Symbiosis with its host *Hemiramphus brasiliensis* (Pisces: Hemiramphidae). *J Crustacean Biol* **26**(3):283–294. doi:10.1651/C-2573.1.
- Behera PR, Ghosh S, Pattnaik P, Rao MV. 2016. Maiden occurrence of the isopod, *Norileca indica* (H. Milne Edwards, 1840) in pelagic and demersal finfishes of Visakhapatnam waters along north-west Bay of Bengal. *Indian J Geo-Mar Sci* **45**(7):856–862.
- Bharadhirajan P, Gopalakrishnan A, Raja K, Murugan S, Vijayakumar R, Rahman MM. 2013. Prevalence of copepod parasite (*Lernaenicus polynemi*) infestation on *Eleutheronema tetradactylum* from Pazhayar coastal waters, southeast coast of India. *J Coast Life Med* **1**(4):278–281. doi:10.12980/JCLM.1.2013D154.
- Bruce NL. 1990. The genera *Catoessa*, *Elthusia*, *Enispa*, *Ichthyoxenus*, *Indusa*, *Livoneca* and *Norileca* n. gen. (Isopoda, Cymothoidae), crustacean parasites of marine fishes, with descriptions of eastern Australian species. *Rec Aust Mus* **42**(3):247–300.
- Brusca RC, Gilligan MR. 1983. Tongue replacement in a marine fish (*Lutjanus guttatus*) by a parasitic isopod (Crustacea: Isopoda). *Copeia* **1983**:813–816. doi:10.2307/1444352.
- Bunkley-Williams L, Williams EH Jr. 1998. Isopods associated with fishes: a synopsis and corrections. *J Parasitol* **84**:893–896.
- Bush AO, Lafferty KD, Lotz JM, Shostak AW. 1997. Parasitology meets ecology on its own terms. *Margolis et al. revised*. *J Parasitol* **83**:575–583.
- Cressey RF, Collette BB, Russo JL. 1983. Copepods and scombrid fishes: A study in host-parasite relationships. *Fishery bulletin United States, National Marine Fisheries Service*.

- Cruz-Lacierda ER, Nagasawa K. 2017. First record of *Norileca indica* (Isopoda, Cymothoidae) parasitic on *Selar crumenophthalmus* and *Decapterus kurroides* (Perciformes, Carangidae) in the Philippines. *Comparative Parasitology* **84**(1):60–63. doi:10.1654/1525-2647-84.1.60.
- DeMeeüs T, Marin R, Renaud F. 1992. Genetic heterogeneity within populations of *Lepeophtheirus europaeensis* (Copepoda, Caligidae) parasite on to host species. *Int J Parasitol* **8**:1179–1181.
- DeMeeüs T, Raibaut A, Renaud F. 1993. Speciation and specificity in parasitic copepods: caligids of the genus *Lepeophtheirus*, parasites of flatfish in the Mediterranean. In: Boxshall GA, Defaye D, eds., *Pathogens of wild and farmed fish: sea lice*. Ellis Horwood, Chichester, pp. 143–150.
- Dipanjan R, Santanu M, Anil M. 2016. First report of parasitic isopod *Norileca indica* Milne-Edwards, 1840 from Northern part of East Coast of India. *International Journal of Experimental Research and Review* **4**:19–25.
- Fogelman RM, Grutter AS. 2008. Mancae of the parasitic cymothoid isopod, *Anilocra apogonae*: early life history, host-specificity, and effect on growth and survival of preferred young cardinal fishes. *Coral Reefs* **27**:685–693. doi:10.1007/s00338-008-0379-2.
- Fogelman RM, Kuris AM, Grutter AS. 2009. Parasitic castration of a vertebrate: effect of the cymothoid isopod, *Anilocra apogonae*, on the five-lined cardinalfish, *Cheilodipterus quinquelineatus*. *Int J Parasitol* **39**:577–583. doi:10.1016/j.ijpara.2008.10.013.
- Froese R, Pauly D. 2018. FishBase, version January, 2018. World Wide Web electronic publication Retrieved from <http://www.fishbase.org>. Accessed dd mm year.
- Ghani N. 2003. Isopod parasites of marine fishes of Pakistan. *Pak Con Z* **23**:217–221.
- Helna AK. 2016. Studies on growth and reproduction of parasitic crustaceans infesting scombrid fishes along the Malabar Coast. PhD dissertation, Kannur University.
- Javed W, Yasmeen R. 1999. A new parasitic isopod of the genus *Norileca* (Crustacea, Cymothoidae) from the Arabian Sea. *Pakistan Journal of Marine Sciences* **8**(1):55–60.
- Kottarathil HA, Kappalli S. 2019. Reproductive system in the male phase of a parasitic isopod (Crustacea) – morphological, histological and ultrastructural evidence for sequential protandrous hermaphroditic changes. *Zool Stud* **58**:4. doi:10.6620/ZS.2019.58-04.
- Kumar AA, Rameshkumar G, Ravichandran S, Nagarajan R, Prabhakaran K, Ramesh M. 2017. Distribution of isopod parasites in commercially important marine fishes of the Miri coast, East Malaysia. *J Parasit Dis* **41**(1):55–61. doi:10.1007/s12639-016-0749-6.
- Leonardos I, Trilles JP. 2003. Host-parasite relationships: occurrence and effect of the parasitic isopod *Mothocya epimerica* on sand smelt *Atherina boyeri* in the Mesolongi and Etolikon Lagoons (W. Greece). *Dis Aquat Organ* **54**:243–251. doi:10.3354/dao054243.
- Madhupratap M, Nair KNV, Gopalakrishnan TC, Haridas P, Nair KKC, Venugopal P, Gauns M. 2001. Arabian sea oceanography and fisheries of the west coast of India. *Curr Sci India* **81**(4):355–361.
- Marcogliese 1991. Seasonal occurrence of *Lernaea cyprinacea* on fishes in Belews Lake, North Carolina. *J Parasitol* **77**(2):326–327.
- Margolis L, Esch GW, Holmes JC, Kuris AM, Schad GA. 1982. The use of ecological terms in parasitology (Report of an ad hoc Committee of the American Society of Parasitologists). *J Parasitol* **68**:131–133. doi:10.2307/3281335.
- Milne-Edwards H. 1840. Histoire naturelle des Crustacés: comprenant l'anatomie, la physiologie et la classification de ces animaux. Librairie Encyclopédique Roret, Paris, sIII, 605 pp. doi:10.5962/bhl.title.16170.
- Moranda S, Sorcib G. 1998. Determinants of life-history evolution in nematodes. *Parasitol Today* **14**:193–196.
- Morton B. 1974. Host specificity and position on the host in *Nerocila phaeopleura* Bleeker (Isopoda, Cymothoidae). *Crustaceana* **26**:143–148.
- Nagasawa K, Petchsupa N. 2009. *Norileca indica* (Isopoda, Cymothoidae) parasitic on bigeye scad *Selar crumenophthalmus* in Thailand. *Biogeography* **11**:131–133.
- Neeraja T, Tripathi G, Shameem U. 2014. Occurrence of the isopod, *Norileca indica* (Isopoda: Cymothoidae) on bigeye scad, *Selar crumenophthalmus* (Bloch) off Mumbai coast, India. *Indian J Fish* **61**:49–56.
- Ohtsuka S, Takami I, Maran BAV, Ogawa K, Shimono T, Fujita Y, Asakawa M, Boxshall GA. 2009. Developmental stages and growth of *Pseudocaligus fugu* Yamaguti, 1936 (Copepoda: Siphonostomatoida: Caligidae) host-specific to Puffer. *J Nat Hist* **43**:1779–1804.
- Ostlund-Nilsson S, Curtis L, Nilsson GE, Grutter AS. 2005. Parasitic isopod *Anilocra apogonae*, a drag for the cardinal fish *Cheilodipterus quinquelineatus*. *Mar Ecol Prog Ser* **287**:209–216.
- Panakkool-Thamban A, Kottarathil HA, Kappalli S. 2016. Branchial cymothoids infesting the marine food fishes of Malabar Coast. *J Parasit Dis* **40**(4):1270–1277. doi:10.1007/s12639-015-0666-0.
- Papapanagiotou EP, Trilles JP. 2001. Cymothoid parasite *Ceratothoa parallela* inflicts great losses on cultured gilthead sea bream *Sparus aurata* in Greece. *Dis Aquat Organ* **45**:237–239. doi:10.3354/dao045237.
- Pattipeiluhu SM, Gill ME. 1998. Ectoparasites of coral reef fishes and their value as biological indicators of pollution. *Cakalele* **9**:25–29.
- Rajkumar M, Perumal P, Trilles JP. 2005. *Cymothoa indica* (Crustacea, Isopoda, Cymothoidae) parasitizes the cultured larvae of the Asian seabass *Lates calcarifer* under laboratory conditions. *Dis Aquat Organ* **66**:87–90. doi:10.3354/dao066087.
- Ramakrishna G. 1980. Techniques of Collection and Preservation of Parasitic Crustaceans. In: *Proceedings of Workshop-Techniques in Parasitology, Zoological Survey of India*, pp. 109–113.
- Rameshkumar G, Ramesh M, Ravichandran S, Trilles JP, Subbiah S. 2014. New record of *Norileca indica* from the west coast of India. *J Parasit Dis* **39**:712–715. doi:10.1007/s12639-014-0416-8.
- Rameshkumar G, Ravichandran S. 2015. First occurrence of *Norileca triangulata* (Crustacea: Isopoda: Cymothoidae) from Indian marine fishes. *J Parasit Dis* **39**:33–36. doi:10.1007/s12639-013-0274-9.
- Rameshkumar G, Ravichandran S, Sivasubramanian K. 2013b. Invasion of parasitic isopods in marine fishes. *J Coast Life Med* **1**:99–105.
- Rameshkumar G, Ravichandran S, Sivasubramanian K, Trilles JP. 2013a. New occurrence of parasitic isopods from Indian fishes. *J Parasit Dis* **37**:42–46. doi:10.1007/s12639-012-0128-x.
- Reiczigel J, Rózsa L. 2005. *Quantitative Parasitology 3.0*. Budapest. Distributed by the authors.
- Richardson H. 1910. Marine isopods collected in the Philippines by US fisheries steamer Albatross in 1907–1908. Department of Commerce and Labor, Bureau of Fisheries Document **736**:1–44.
- Rijin K, Sudha K, Vineesh PJ, Anilkumar G. 2019. Seasonal Variation in the occurrence of Parasitic Isopods and Copepods (Crustacea) Infesting the Clupeidae fishes of Malabar Coast, India. *Turk J Fish Aquat Sc* **19**:241–249. doi:10.4194/1303-2712-v19_03_07.
- Rokicki J. 1982. *Lironeca indica* Edwards, 1840 (Crustacea,

- Isopoda) from *Selar criimenophthialmiis* (Bloch). *Wiadomosci Parazitologiczne* **38**:205–206.
- Romestand B, Trilles JP. 1979. Influences des Cymothodiens *Meinertia oestroides*, *Meinertia parallela* et *Anilocra physodes* (Crustacea, Isopodes; parasites de poissons) sur la croissance des poissons hôtes *Boops boops* et *Pagellus erythrinus* (Sparides). *Z Parasitenk* **59**:195–202.
- Saarinen M, Taskinen J. 2005. Local adaptation in a crustacean parasite–molluscan host interaction: a field experiment. *Ecol Ecol Res* **7(8)**:1191–1199.
- Saravanakumar A, Balasubramanian T, Raja K, Trilles JP. 2012. A massive infestation of sea snakes by cymothoid isopods. *Parasitol Resr* **110(6)**:2529–2531. doi:10.1007/s00436-011-2795-4.
- Schioedte JC, Meinert F. 1884. *Symbolae and Monographiam Cymothoarum Crustaceorum Isopodum familiae. IV. Cymothoidae. Trib. II. Cymothoinae. Trib. III. Livonecinae.* *Naturhistorisk Tidsskrift series III* **14**:221–454.
- van der Wal S, Smit NJ, Hadfield KA. 2017. Redescription and Molecular Characterisation of the Fish Parasitic Isopod *Norileca indica* (Milne Edwards, 1840) (Crustacea: Isopoda: Cymothoidae) with a Key to the Genus. *Afr Zool* **52(3)**:163–175. doi:10.1080/15627020.2017.1382389.
- Smit, NJ, Bruce NL, Hadfield KA. 2014. Global diversity of fish parasitic isopod crustaceans of the family Cymothoidae. *Int J Parasitol Parasites Wildl* **3(2)**:188–297. doi:10.1016/j.ijppaw.2014.03.004.
- Sudha K, Anilkumar G. 1996. Seasonal growth and reproduction in a highly fecund brachyuran crab *Metopograpsus messor* (Forsk.) (Grapsidae). *Hydrobiologia* **319**:15–21.
- Sudha K, Supriya NT, Anilkumar G, Chang ES. 2012. Hemolymph Ecdysteroid titres in a brachyuran crab (*Uca triangularis*) that concomitantly undergoes molting and reproduction. *Zool Stud* **51**:966–976.
- Syama VP, Supriya NT, Sudha K, Anilkumar G. 2010. Seasonal growth and reproduction in two brachyuran species inhabiting diverse ecosystems. *In: Gupta Verma VKAK, Singh JD (eds.) Perspectives in Animal Ecology and Reproduction.* Daya Publications, Vol. VI, New Delhi, pp. 275–289.
- Trilles JP. 1968. *Recherches sur les Isopodes Cymothoidae des côtes Françaises.* Dissertation, University of Montpellier France, N°enregistrement CNRSO, 2305,1: Bionomie et Parasitisme et Vol. 2: Biologie générale et sexualité, 793 pp. Deuxième thèse, Systématique et Faunistique, 181P.
- Trilles JP. 1969. *Recherches sur les isopods “Cymothoidae” des côtes françaises. Aperçu général et comparative sur le bionomie et la sexualité de ces crustacés (Researches on the isopod Cymothoidae of French coasts. General and Comparative Overview on bionomics and the sexuality of these crustaceans).* *Bulletin de la Société zoologique de France* **94(3)**:433–445.
- Trilles JP. 1972. *Les Cymothoidae (Isopoda, Flabellifera) du Muséum national d’histoire naturelle de Paris. Etude critique accompagnée de précision en particulier la répartition géographique et l’écologie des différentes espèces représentées. I. Les Ceratothoinae Schioedte et Meinert, 1883.* *Bulletin du Muséum national d’histoire naturelle, Paris, 3e sér., n° 91, Zool.* **70**:1231–1268.
- Trilles JP. 1976. *Les Cymothoidae (Isopoda, Flabellifera) des collections du Muséum National d’Histoire Naturelle de Paris. IV. Les Lironecinae Schioedte & Meinert, 1884.* *Bulletin du Muséum National d’Histoire Naturelle, Paris 3ème série* **390, Zool.** **272**:773–800.
- Trilles JP. 1979. *Les cymothoidae (Isopoda, Flabellifera, parasites de poissons) du Rijksmuseum van Natuurlijke Historie de leidern II. Afrique, Amerique et regions indoouest-pacifiques.* *Zoologische Mededelingen (Leiden)* **54**:245–275.
- Trilles JP. 1994. *Les Cymothoidae (Crustacea, Isopoda) du Monde. Prodrôme pour une faune.* *Studia Marina* **21/22(1-2)**:5–288.
- Yamauchi T, Ohtsuka S, Nagasawa K. 2005. Ectoparasitic Isopod, *Norileca indica* (Crustacea, Isopoda, Cymothoidae), obtained from the Stomach of *Coryphaena hippurus* (Perciformes, Coryphaeniadae) in the Philippines. *Biogeography* **7**:25–27.
- Yu H Y, Li XZ. 2003. Study on the Cymothoidae from Chinese waters. *Studia Marina Sinica* **45**:223–238.