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Is *Naegleria fowleri* an Emerging Parasite?

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Abstract

Naegleria fowleri causes an uncommon but deadly disease called primary amoebic meningoencephalitis (PAM). There has been an increase of reported PAM cases, particularly since 2000. Although water is the dominant route of transmission of PAM, infection through soil/dust is a possible alternative route. We have observed differences in epidemiology between the southern US states and the Indian subcontinent (ISC). The patient age range is greater in ISC than the US, and there are more infections in ISC which are not water-associated. We show that PAM is under reported and argue that climate change will increase the incidence of PAM and the geographic range of *N. fowleri* will spread poleward.

Pathogenic amoebae

The obligate parasitic amoeba *Entamoeba histolytica* causes huge human mortality, but the **free-living amoebae (FLA**, see Glossary**)** in the same supergroup amoebozoa, *Acanthamoeba*, *Balamuthia* and *Sappinia,* are known to cause encephalitis in humans [1], while the excavate amoeba *Naegleria fowleri* (Box 1) causes meningoencephalitis [2]. Members of the **amoeboflagellated** genus *Naegleria* are common throughout the world, particularly in soils and freshwater. The genus was named to honour the German protozoologist Kurt Nägler [3] and currently contains about 50 recognised species. Only one of these, *N. fowleri,* named for Malcolm Fowler [4], is a confirmed human pathogen. Fowler and Carter first described the disease [2], which was later named primary amoebic meningoencephalitis (PAM) by Cecil Butt [5] (Box 2). The term "primary" is used as the amoebae are the root cause of the disease, and "meningoencephalitis" is an encephalitis that also involves inflammation of the meninges.

Is *Naegleria fowleri* an emerging pathogen?

An emerging infectious disease is defined as a disease whose incidence has increased in the past 20 years and which seems likely to increase further (CDC Atlanta). *N. fowleri* has been described as an emerging parasite by some [6], and an emerging medical threat by others [7]. We have searched the literature to find as many PAM cases as possible, looking for trends in reported infections. A previous survey of PAM laboratory-confirmed cases in the US between 1962 and 2008 showed variation from year to year but no overall increase in incidence was claimed [8], however looking at the worldwide data over a longer period (Figure 1A) we suggest that there has been an increase in reported cases, especially since 2000. However, we do not know if the incidence of the disease is actually increasing or if the increasing numbers of publications merely reflects its increasing recognition and notoriety, especially since the evocative name "brain-eating amoeba" has been adopted by the media and by some biologists [9]. Whatever the reason, we argue that PAM qualifies for labelling as an emerging infectious disease, since it seems inevitable that its actual incidence will increase due to climate change.

Patterns of primary amoebic meningoencephalitis

Much of what is known about PAM has been gleaned from cases in the USA, but differences between the world's regions are to be expected due to local geography and human habits. We have found a difference in the patterns of PAM victims between the USA and the Indian subcontinent (ISC), USA victims tending to be younger than those from ISC (Figure 1B) with

the exception of victims under 1 years old, for which the recorded rate was 7 times greater in ISC than in the USA. The age spread within the ISC is also greater than the USA population (Figure 1B). The world-wide gender bias shows that there are more male than female cases (Figure 1C) but we have found that this bias is greater in the ISC group, with 14 times more males than females, compared to 3 times more in the USA group. The typical US victim is a young male who becomes exposed to *N. fowleri* in recreational situations. Boys are more likely to play in water and to be more boisterous as they do so, immersing themselves and stirring up lake sediments containing *N. fowleri* **trophozoites** and **cysts**, which explains the gender bias. In the ISC, contact with *N. fowleri* seems to be through play but also through ablution rituals, washing and a lack of chlorination, meaning that the general population is at risk from PAM, not just those who play in natural bodies of water, for which chlorination is not feasible.

"Wet" vs "dry" infection

We calculate that where possible sources of *N. fowleri* have been reported, 93% of PAM cases are described as infections that have resulted from exposure to water (314 cases out of 336). Water is clearly the most frequent route of infection in most cases, but it has been argued that people can also be infected through cyst-laden dust entering the nasal passages and then excysting, leading to infection [10, 11]. These "**dry infections**" are particularly worrying since there is little that people living in such areas can do to avoid cyst inhalation. Infections with cysts (dry infections) account for 6.5% of PAM cases where possible sources are reported. Cysts may also enter the **nares** through the **nasolacrimal ducts** when contaminated dust alights on the eye. Although the dry infection route has been doubted by some [12] who point out that *N. fowleri* cysts last about 5 minutes when desiccated [13], we have found that amoeba cysts survive for years when dried in fine clay [14], and so it is likely that fine clay improves cyst survival in air. We have found too that the dry infections tend to be in warm/hot regions (Figure 2). The incidence of nasal carriage, which is discussed later, is fortunately higher than the development of PAM, suggesting that there may be a threshold effect in which large numbers of activated amoebae are required to penetrate the epithelium and invade the brain, so that a low cyst carriage does not represent a high risk. Physical trauma to the nasal epithelium may also be important in PAM development through dry infection. Another marked difference between PAM cases from the USA and the ISC is that the latter are more likely to have been suspected dry infections (0% in USA *vs* 8% in ISC).

Where is *N. fowleri* and where is PAM?

N. fowleri is known to be distributed throughout all continents except Antarctica and is especially common in the warmer equatorial countries [15]. In temperate countries such as those in Northern Europe, *N. fowleri* is typically found in waters that are warmed either artificially (*e.g.* cooling towers from power stations) or naturally (*e.g.* **geothermal** areas). Cases of PAM are typically restricted to warm countries and their distribution correlates most strongly with areas where average annual temperatures lie between 15 – 18ºC (Figure 2). Many have reported that PAM is highly seasonal away from equatorial regions [16]. The distribution of PAM largely reflects that of human populations within the warmer regions, although the ISC and Southeast Asia are notable exceptions, as here higher PAM incidence is found together with high temperatures. Many of the European recorded cases are not recent and have occurred in natural warm springs (*e.g.* City of Bath, England), or artificially heated areas such as power stations and baths (Belgium, Czech Republic), however more recent cases in Minnesota (white arrow, Figure 2) occurred far from other cases and in a natural lake, leading to concerns that *N. fowleri* may now be occurring further north due to climate change [17]. Another northerly case from N.E Pennsylvania [18] has been questioned [19].

It is important to understand what determines the distribution and abundance of *N. fowleri* in the environment and which niches these amoebae occupy within the ecosystems. This is known to be influenced by factors such as temperature, salinity, the availability of suitable prey organisms and the presence of amoeba pathogens, including some bacteria and viruses. A further, less-explored consideration is that of competition with other amoebae, especially other *Naegleria* species [20].

Temperature

N. fowleri is associated with elevated temperatures (Figures 2 & 3). It is found in regions where water/soils are warm, such as geothermal sources, or where human activities result in water bodies that are warm over extended periods. *N. fowleri* grows at temperatures of 30º - 46˚C [21], but in **biofilms** it is reported that the amoebae need temperatures of 42ºC to support growth and that 32ºC was insufficient [22]. Amoebae remained viable for 24hrs at 49°C but were nonviable by 48 h [23]. Trophozoites and *N. fowleri* cysts can also survive for a few minutes to hours at 50-65°C, with cysts being more resistant to the effects of high temperatures than the trophozoites are [13]. *N. fowleri* trophozoites degenerate within hours below 10ºC [13], while the cysts can survive at 4ºC for six months [24] meaning that they can potentially overwinter in lakes/rivers and grow the following summer.

Salinity, osmotic tolerance

N. fowleri must be capable of tolerating human physiological ion concentrations to survive passage through the **cribiform plate** into the brain. The tonicity of human tissues is equivalent to 0.9% NaCl, and *N. fowleri* trophozoites are reported to remain viable up to 1.4% [23] or even 2% [25]. It therefore seems that *N. fowleri* is quite capable of adapting to survive the salinity of human tissue. Fresh-water amoebae adapt to higher salinity over time [26] and because the period of exposure and expression of disease symptoms is so short, this adaptation probably takes place with the individual amoebae causing the infection rather than their progeny. In mice, *N. fowleri* trophozoites are found within the olfactory bulb as early as 24hr after nasal inoculation [27].

The distribution of *N. fowleri* in water bodies

The availability of suitable prey is likely to be an important determinant of *N. fowleri* distribution in warm lakes (Figure 4). FLA are found in large numbers in floating biofilms at the meniscus with bacteria [28] and *N. fowleri* are enriched here too [29, 30]. **Cyanobacteria** are extensively grazed by protistans [31] especially amoebae [32]. *Naegleria* (and other FLA) are associated with cyanobacteria-dominated layers in a stratified lake [33] and *N. australiensis* is known to prey on a range of cyanobacteria (but not all) [34]. Cyanobacterial blooms can maintain a position in the water column as they can regulate their buoyancy through gas vesicles and trophozoites are able to crawl amongst these floating masses as they prey upon them. While *N. fowleri* trophozoites are found associated with these bacteria in the water column, free swimming *N. fowleri* **flagellates** are also found here. Amoebae prey on bacteria in two-dimensional biofilms much more efficiently than they can on cells in suspension [35] and the *N. fowleri* flagellate cannot feed at all, so that it is expected that much of their feeding and growth takes place on the lake floor within the sediment. **Groundwater** systems occupy vast volumes with huge biofilm potential, often with elevated temperatures. In Arizona *N. fowleri* has been found in groundwater [36], and the temperature varied between 13 and 46ºC in the wells that draw on this water source [37, 38]. In Australia, too, the amoeba is found in groundwater and where this breaks the surface, such as in **mound springs** and bore holes [39]. Groundwater may well be the largest natural niche for *N. fowleri* and the most important reservoir for re-infection of surface waters (Figure 4).

Climate change and the rise of *N. fowleri*

Climate models predict that almost everywhere will become warmer, and a global rise in average temperatures has already been recorded. It is also predicted [40] that large areas will experience increased drought conditions (USA, Central America, Brazil, North Africa, the Mediterranean region, Australia, South China). Other areas (Northern Europe, Central East Africa, the Indian subcontinent, Northern Eurasia) will see increased precipitation which will cause increased erosion and eutrophication of freshwater courses. Perhaps counterintuitively both drought and increased precipitation may favour *N. fowleri* and the spread of PAM, through different mechanisms. Drought may make the use of rooftop rainwater systems more attractive in some areas and in others the use of artesian wells drawing on infected ground-water is expected to increase the risk of *N. fowleri* exposure and PAM. Concerns around the microbiological safety of roof-harvested rainwater have already been raised [41], and *N. fowleri* is often detected in roof-harvested rainwater tanks in Australia and South Africa [42]. Although there is an abundance of evidence to suggest that global warming is real, does this actually mean that the habitat favoured by *N. fowleri* is increasing together with a population increase? This amoeba seems to have an advantage over related organisms when temperatures are above 30°C (Figure 3). Many claims have been made in the literature of a connection between global warming and PAM [7, 16, 43,]. Given the thermophilic nature of *N. fowleri* this is an attractively simple idea, but what is the evidence for this connection? It has been observed that in the US, the rate of PAM is distinctly seasonal [8,16] and this has also been reported in Australia [12] and in Karachi [44], but here screening for PAM is routinely undertaken only in the summer months, so the observed seasonality may have been exaggerated in Karachi. Several cases of PAM specifically mention that the patients had sought relief from the heat by immersing themselves in the water that has been blamed for their subsequent infection [45]. In general, the seasonality of PAM suggests a causal link between higher temperatures and greater incidence of disease, as indicated too by the geographic occurrences.

Are we only seeing the tip of a PAM iceberg?

There is a strong suspicion that PAM is very much more common than currently indicated, especially in the developing world where conditions for *N. fowleri* tend to be favourable and where PAM maybe masked by a myriad of other diseases and by economic deprivation. PAM is under-reported, as many cases are misdiagnosed as viral or bacterial meningoencephalitides and autopsies are not always carried out, either through lack of time or because family members deny permission [46]. With approximately 3 PAM cases typically reported annually in the

USA, it is estimated that there are likely to be a further 13 unreported cases [47]. Although PAM is considered a rare disease, it has been associated with outbreaks involving many deaths, for example, 16 individuals fell victim to PAM from a single swimming pool in what is now the Czech Republic [48] and an outbreak in Karachi during 2015 – 2017 has claimed at least 24 lives [49]. It is probable that, particularly in the developing world, many more such outbreaks have occurred and have gone unreported in the biomedical literature due to a lack of expertise in local medical staff [50]. A further reason why incidences of PAM may be high in the hot dry regions of Africa for example is the presence *N. fowleri* in the nares (nasal passages) of non-infected people.

Assessing the risk of PAM from nasal *N. fowleri* carriage

Although it is reported that the most common route of entry into the human host is through the nose by contact with trophozoite- laden water (**wet infection**), it is also possible that dry cysts in dust can infect (dry infection) [51]. Two cases from Northern Nigeria arose in the very dry conditions of the **harmattan season** [11], while *N. fowleri* was recovered from the air during the harmattan [52] and have been isolated from the nares of 2 out of 50 non-infected children during the hartmattan in Zaria, Nigeria [53]. Additionally, a separate study some 400 miles away, in Borno State, Nigeria, found 3 out of 50 children carrying viable *N. fowleri* in their nares in [54], while none were found in the nares of 1039 people from the Brest region of Brittany, France [55], none in 262 students from Flinders, southern Australia [56], none in 1,551 army recruits from North Bohemia [57] and none in 500 children in Alexandria [58]. It seems highly probable that carriage of *N. fowleri* in the nares increases the risk of PAM, so that the incidence of the disease is likely to be very much greater in warm, dry regions such as Nigeria and India (Figure 2).

Concluding remarks

Many countries are already recording extreme temperatures and many people are seeking relief from the heat by immersing themselves in water that is often very warm and polluted by **coliforms**, perfect conditions for the growth of *N. fowleri*. People in some of the same areas practise cleansing rituals in which water is forced through the nares by specialised **neti pot** devices or other means. This is believed to be the cause of the alarming increase in the incidence of PAM in cities such as Karachi [7, 59]. However, it is very likely that other cities are also

experiencing silent outbreaks and that Karachi has become a known hotspot for PAM solely because of local expertise in PAM diagnoses. Sensitive education is needed to raise awareness of PAM, in the hope that simple alterations in practice, such as the use of water which has been boiled then cooled for neti pots, may prevent *N. fowleri* infections. We cannot presently state with certainty that *N. fowleri* is an actual emerging pathogen since we do not know unequivocally that the actual number of infections is increasing or has increased over the past 20 years, although this remains a distinct possibility (see Outstanding Questions). There does seem to be good reason to suspect that the number of *N. fowleri* cases will increase as the human population approaches its expected peak, especially in some of the regions where conditions seem most suitable for *N. fowleri* growth (UN Population Division 2017) and so the label of emerging pathogen is warranted on those grounds alone. We expect that the gender imbalance in cases will remain. Other free-living thermophilic amoebae, such as *Balamuthia mandrillaris* and some *Acanthamoeba* strains, also cause encephalitis and these too are expected to increase as temperatures increase, making awareness of pathogenic amoebae in general more important. A better understanding of the parameters that dictate the presence and abundance of the pathogens is needed. It is also clear that better options for their diagnosis, treatment and eradication are required.

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Glossary

Amoeboflagellate: An amoeba that can transform reversibly into a flagellate. The genus *Naegleria* is a well-known amoeboflagellate.

Biofilm: A biofilm is a layer of cells usually dominated by bacteria which associate on a surface. An extracellular layer of proteins and polysaccharides is often produced by the community which may be grazed by eukaryotes such as amoebae which prefer to feed on surfaces.

Cerebrospinal fluid (CSF): The liquid percolating the brain and spinal cord.

Coliform: A group of gram-negative rod-shaped bacteria that ferment lactose. Faecal *coliforms* are *coliforms* typically present in the gut and faeces of birds and mammals.

Cribiform plate: A sieve-like extension of the ethmoid bone which supports the olfactory bulb of the brain.

Cyanobacteria: A group of photosynthetic bacteria that produce oxygen. They were known as the blue green algae although this term is now disfavoured as they are not algae.

Cyst: A resting non-dividing stage formed by many protists including *Naegleria*. The *Naegleria* cyst is usually has a single nucleus and a protective cell wall.

Dry infection and wet infection: A wet infection is defined by the patient receiving *N. fowleri* infection from an aqueous source introduced directly to the nares. A dry infection is defined as *N. fowleri* being introduced to the nares probably as cysts within inhaled dust or sand. For each PAM case, we have reviewed the literature and classified them as being either wet (usually) or dry where there is sufficient information as to how the disease was acquired. In most cases the source cannot be absolutely verified but we have been guided by the author's opinion as to the most likely source or situation. While the majority of cases are wet, it is important not to exclude the possibility that dry infections can occur under specific conditions.

Flagellate: A cell that is capable of swimming through the possession of one or more flagella. The *Naegleria fowleri* flagellate stage typically has two flagella.

Free-living amoebae (FLA): Those amoebae capable of living without parasitizing metazoans but some of which have that ability. The term is used principally to differentiate this group from others such as Entamoebae (predominantly obligate parasites).

Fulminant: In this context a disease of rapid onset and escalation. From Latin fulmināre, to strike with lightning.

Geothermal: Heat originating from the Earth's core produced by radioactive decay

Groundwater: The water that lies beneath the Earth's surface in voids of various sizes between soil particles, in rock fissures and is subterranean lakes.

Harmattan season: The name given to the season between November and March in West Africa when dry and dusty air blows from the Sahara Desert.

Mound springs: Primarily an Australian phenomenon in which distinctive mounds are pushed up by springs gushing to the surface from groundwater.

Nares: The openings of the nose and nasal cavity

Nasolacrimal ducts: Also called the "tear duct" they carry tears from the eye to the nasal cavity.

Neti pot: A devise used to facilitate the entry of water into the nares in an act of personal hygiene, as a religious act or both.

Trophozoite: The actively moving amoeboid stage of the organism.

457 **Box 1. *Naegleria fowleri*, the pathogen**

458 As is typical for the genus, *N. fowleri* exists in three distinct phases (Figure I): the resting cyst,
459 a swimming flagellate and the active multiplying amoeba stage, also called the trophozoite
460 [60]. Only the latter feeds and can invade the human host, although the flagellate is sometimes
461 observed in **cerebrospinal fluid (CSF)** [45]. In active locomotion, the trophozoite is about
462 22µm in length and 7µm wide and the locomotory rate is about 45µm /min at 37ºC [4]. The
463 nucleus has a distinctive appearance with concentric nucleolus (inner) and karyosome (outer)
464 regions. The flagellate is pear-shaped and 15µm long, with two apical flagella around 12µm in
465 length. The presence of the flagellate stage has been used with other features to identify *N.*
466 *fowleri* [61], but such identification is unsafe, as many strains do not produce flagellates under
467 the conditions of this test [61, 62]. The cysts are between 7 and 15 µm in diameter and unlike
468 some other *Naegleria*, do not have visible pores in the wall (although they have been observed
469 under electron microscopy). The nucleus is often not visible in the cysts of *N. fowleri*, whereas
470 it is in other *Naegleria*. Figure I shows how the stages are related to each other and the
471 approximate times taken to transform between them under conducive conditions. *N. fowleri*
472 can be differentiated from other FLA in brain tissue. *Acanthamoeba* is only rarely found in the
473 CSF [1] and *Acanthamoeba* cysts are frequently seen in human brain tissue while *N. fowleri*
474 cysts are not typically seen [63].

475

476 **Figure I (in Box 1). The life cycle of *Naegleria fowleri*.** The trophozoite at amoeboid stage
477 is motile through its actin-based cytoskeleton and crawls around eating bacteria (or host cells
478 in its parasitic mode). This is the reproductive stage and it can differentiate into a flagellate, or
479 a cyst. The flagellate can swim through its two (usually) flagella that are microtubule-based.
480 The cyst is a resting stage which is adopted during stressful situations such as suboptimal
481 temperatures or lack of prey. The time taken to differentiate between these stages are shown.

482

483 **Box 2. Primary amoebic meningoencephalitis (PAM), the disease.**

484 PAM is usually described as being a rare disease and it is certainly rarely reported, as we have
485 been able to find only 431 cases in the literature. However, there is a tendency for PAM to
486 occur in outbreaks. 16 cases arose in one outbreak in a swimming pool [48] and 3 lethal PAM
487 cases are suspected and only one confirmed over a 14-year period, in a "tiny population"
488 centred around a Queensland cattle station [39]. These examples attest both to the under-
489 diagnosed nature of PAM and to the persistence of the parasite in areas that suit it. The
490 supposed rarity of the **fulminant** disease PAM must also be set against its depressingly high

491 mortality rate, and it is still usually diagnosed *post-mortem* [64]. There is a risk that outbreaks
492 larger than those already documented may occur if vigilance fails.
493 Symptoms and diagnosis of PAM Patients often present with headaches, sore throat, blocked
494 nose, fever, nausea, altered taste and smell perception and photophobia [59, 65]. Projectile
495 vomiting may occur [46], caused by increased intracranial pressure activating the *area*
496 *postrema* of the *medulla oblongata*. Lethargy and irritability often subside into
497 unconsciousness and coma. PAM can easily be mistaken for other meningitises, and the
498 symptoms of PAM and bacterial meningitis are very similar [66]. An early diagnosis is
499 essential because of the very rapid development of the infection. High-powered microscopes
500 are necessary to differentiate the motile amoebae in CSF from neutrophils and this should be
501 done as soon as possible after lumber puncture, as the amoebae quickly degenerate outside the
502 body unless treated carefully [67]. Skill is required to culture the amoebae from clinical
503 samples, and this is a time-consuming activity. PCR diagnosis is effective if the correct primers
504 are immediately available, but this is usually used to confirm a lethal infection rather than to
505 guide clinical practise in treatment.
506 PAM is usually contracted through exposure to water, when the amoebae contact the nasal
507 epithelium of the human host. The incubation period after exposure is 3-8 days, after which the
508 disease develops rapidly, the patient typically dying 7-10 days after the first appearance of
509 symptoms. There is no indication that PAM is infective between humans, although lab
510 experiments suggest that mouse-to-mouse transmission of *N. fowleri* may occur [68].
511 A 95% mortality rate is often quoted for PAM [69] and we have found a rate of 94.6% based
512 on the 431 cases analysed here, however among the 21 cases in which PAM was successfully
513 treated, there are some in which the involvement of *N. fowleri* is questionable. For example, in
514 the surviving PAM case in Allahabad, Pakistan, *N. fowleri* was only identified microscopically
515 [70], this was also true in other apparently successful cases [71]. If we remove those apparently
516 successful cases where there is reason to doubt the identity of the pathogen, the mortality rate
517 rises to 96.4% for the cases analysed here.
518
519

520 **Figure Legends**

521 **Figure 1. Global PAM status**. **A**. Numbers of reported PAM cases from the literatures world-
522 wide between 1961-2018. Years are from either when cases were recorded at hospitals, or in
523 the absence of this information, the years of the publication of the report. The incidence of
524 reports shows an increasing trend after around 2000 in world-wide totals whereas the USA
525 report shows a less marked increase. **B**. USA age profile (blue) vs Indian subcontinent (ISC)
526 age profile (orange) of PAM cases. There are more old victims from ISC than from the USA.
527 **C**. World-wide gender and age bias. There are more male cases (blue) than female case
528 (orange) but the overall age distribution is similar.

529

530 **Figure 2. Worldwide distribution of reported PAM cases.** Cases are clearly limited to the
531 warmer parts of the world, but the extremely warm areas tend to show less cases in line with
532 previous reports [15]. A recent northerly case [17] (white arrow) may suggest an expansion to
533 the range in the US. However, it is noticeable that the dry infections (blue dots) appear to be
534 associated with warmer areas than the wet infections (black dots). The atlas shows the annual
535 average surface temperature (°C). Used by permission of The Center for Sustainability and the
536 Global Environment, Nelson Institute for Environmental Studies, University of Wisconsin-
537 Madison.

538

539 **Figure 3 The temperature range of *Naegleria* sp..** Note that the lower range for growth tends
540 to be less well characterised than the upper temperature.

541

542 **Figure 4. A summary of the habitats occupied by *N. fowleri*.** The amoebae flourish in warm
543 ground water bodies [39]. *N. fowleri* exists in the warm groundwater in all three forms. The
544 groundwater is taken to the surface by boreholes or by natural areas such as Australian mound
545 ponds to infect warm rivers and lakes. Amoebae on the surface transform into cysts which may
546 then be infective, especially when airborne. Amoebae may exist in the surface biofilm, in
547 association with mid-water buoyant cyanobacteria and on and in the sediment. Flagellates are
548 found throughout the water column.

549

550