Reef Focus: Marine "White Spot": Cryptocaryonosis

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Perhaps due to its lethality and a lack of efficacious remedies for reef aquaria, numerous articles exploring the natural history and mitigation of cryptocaryonosis have been authored for the hobbyist. Herein we strive to present the most comprehensive which equips both domestic and commercial aquarists with the means by which they can *safely* eradicate this and many other pathogens in recirculating *fish-only* environments. Benefit from a deep delve into some pertinent fish physiology and possible natural remedies whilst becoming accustomed to some unfamiliar terms.

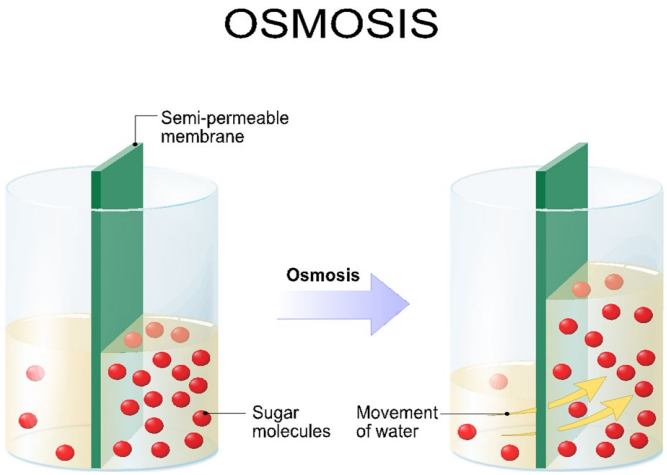

Fig 1. The gravity-defying movement of water (osmosis) through a sugar-impenetrable membrane from the weaker to the stronger solution to equilibrate ionic strengths.

Water is a universal and polar solvent while solutes are dissolved in solvents to create solutions. Salts and gases are solutes, whilst saltwater is an aqueous solution.

Osmosis describes the movement of water through a solute-impenetrable yet semipermeable membrane from a region of low solute concentration to a region of higher: water moves through the membrane to balance the concentrations of the solutions on either side (Fig 1.).

Osmotic Shock

The solute concentration of a unicellular marine organism's cytoplasm or soluble cellular contents is equilibrated to the specific gravity (SG) of the surrounding water, whilst their cell membrane is semipermeable. When they are therefore, abruptly submerged in freshwater, they suffer osmosis-instigated water ingress which causes swelling and cellular rupture. Likewise, water leaks from freshwater cells placed in seawater which elicits desiccation and collapse. Complex organisms like fish oppose water movement by maintaining or upregulating osmoregulation which expends energy and induces stress (Wood 2015). Freshwater/saltwater baths help to detach/clear some saltwater/freshwater parasites; however, such practices perturb the communities of their skin's mucosal microbiome which is the fish's first line of defence (Banner, personal communication). Furthermore, osmotic shock induces the release of corticosteroids from the fish's adrenal cortex which compromises immunity (Tomasso 1994).

The Function of Chloride Cells

The innards of a saltwater fish are maintained at around 0.9 while it is immersed in 3.5 percent. Hence water is continuously escaping via osmosis. To prevent dehydration, marine fish must continuously drink water, pass only concentrated urine, and actively export chloride ions (Cl^-). A freshwater bony fish's (teleost's) osmoregulation is the reverse, whilst Cl^- are exported in seawater using mitochondrial-rich chloride cells. Meanwhile, sodium ions (Na^+) leave through the intercellular junctions of gills which are comparatively leaky in saltwater fish (Fig 2.).

Keywords: Ciliates; Copper; *Cryptocaryon irritans*; DIY Marine Chemotherapeutic; DIY Marine Cure-Alls; Formalin; Marine "*Ich*"; Marine "White Spot"; Marine Ornamental Teleosts.

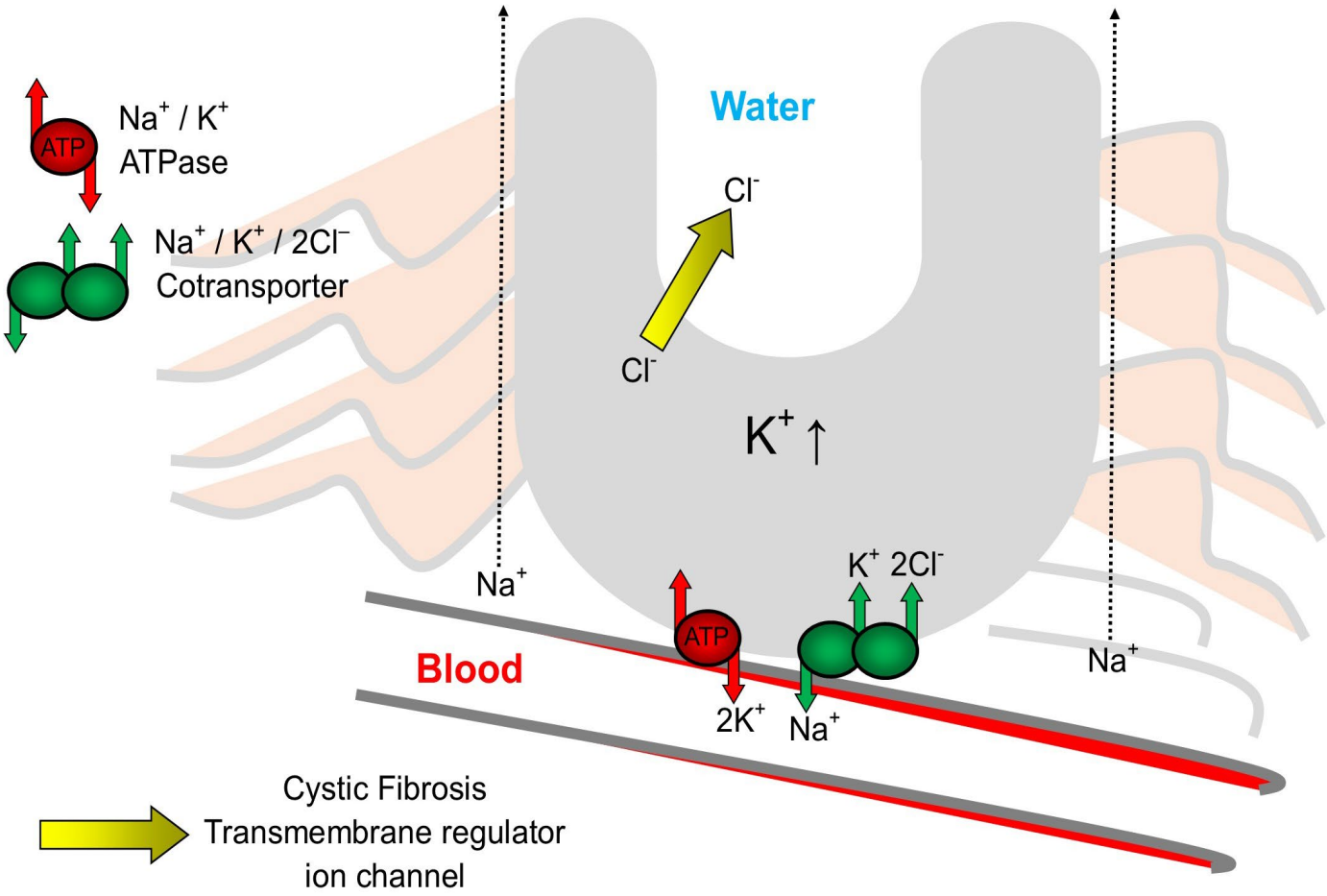

Fig 2. The purging and export of Na^+ and Cl^- in saltwater teleosts using their intercellular spaces and chloride cells. Cotransporters actively internalise Cl^- which leave via the cystic fibrosis transmembrane ion channel. Fortification of intracellular potassium ions (K^+) expedites the diffusion of Na^+, when K^+ are actively transported into blood. Adapted from Marshall 2002, McCormick 2002, and Morera et al. 2015. Chloride ions are also excreted in urine which may be liberated across the gill (Ip & Chew 2010).

Chloride cells internalise Cl^- and K^+ across their basal membrane under normal circumstances, whilst Na^+ are pumped from the cell into the body using cotransporters and exchangers. Intra-chloride cell Na^+ and K^+ are maintained at low and high concentrations, where Cl^- leave the cell via specialised ion channels, whereas Na^+ diffuse through intercellular spaces, but only when K^+ are actively transported into blood (Fig 2.; Marshall 2002; McCormick 2002; Morera et al. 2015).

Features of some Ciliated Chromists

Chromista is a kingdom of single- or multi-cellular often photosynthetic eukaryotes whose plastids contain chlorophyll. Chromists include non-photosynthetic pseudofungi (Cavalier-Smith et al. 2006; Mohammadi et al. 2021), where much of the species-rich phagocytic mixotrophic chromists of which the infrakingdom Heterokonta is conspicuous, were hitherto considered Protozoa of the kingdom Protista (Cavalier-Smith et al. 2006; Cavalier-Smith 2010; Cavalier-Smith 2018). Nevertheless, the national centre for biotechnology information's (NCBI's) taxonomy browser "root", has yet to accept and adopt Cavalier-Smith and colleagues' recommendations. Hence several scientists regard *Cryptocaryon* as protozoa.

Before launching headlong into an exploration of *Cryptocaryon irritans*, we first investigate basic but little-known ciliate physiology. Ciliates are single-celled eukaryotic chromists divided into oral and somatic regions swathed in hair-like processes called cilia (Lynn 1981). Referred to as their infraciliature whose primary role is locomotion, an external microtubule framework supports, animates, and synchronises these hairs. A cilium's microtubule is a basal body or kinetosome (Chapman et al. 2000). A pair of kinetosomes is called a kinetid (Lynn & Small 1982) which can be a singular monokinetid; a pair of dikinetids, or multiple-aligned polykinetids (Lynn 1981). Some kinetosomes do not have cilia (Lom & Nigrelli 1970; Woo 2006; Lynn 2008), whilst an extensive row of kinetids constitutes a kinety (Nelsen & Frankel 1979).

Kinetid can refer to the ciliature of an anatomical feature, like the oral kinetids that direct food into the mouth (buccal cavity), while their infraciliature assists species discernment (Lynn & Small 1982; Chapman et al. 2000; Gong & Song 2006; Chen et al. 2015; Wang et al. 2017).

Nematodesmata are supportive rod-like microtubules that line the cytopharyngeal tube (gullet), where six or more terminate in beak-like armature in the commensal ciliated chromists and opportunistic aetiological agents of brooklynellosis, slime-blotch, or clownfish disease, *Brooklynella hostilis* (Fig 3.; Lom & Nigrelli 1970; Woo 2006; Lynn 2008; McLean et al. 2008), while ingested cellular debris remains evident in food vacuoles (Diamant 1998).

Stomatogenesis

Some ciliates have the means to generate another cell using binary fission. Staining ciliates with silver nitrate highlights their kineties, which facilitates the microscopic examination of the development of a daughter cell (proter) from the maternal opisthe (Nelsen & Frankel 1979). Species of *Brooklynella* are easier to differentiate by virtue of their reduced infraciliature (Lom & Corliss 1971), whereas opisthe cloning and proter generation (transverse binary fission) can be observed from the replication boundary. The oral primordium clones the nascent mouth and somatic cells prior to daughter separation; however, stomatogenesis of the proter cannot be observed in "real-time", and thus researchers require mixed cultures of ciliates in various vegetative states (Fig 4.; Wise 1965; Gong & Song 2006; Aufderheide 2016).

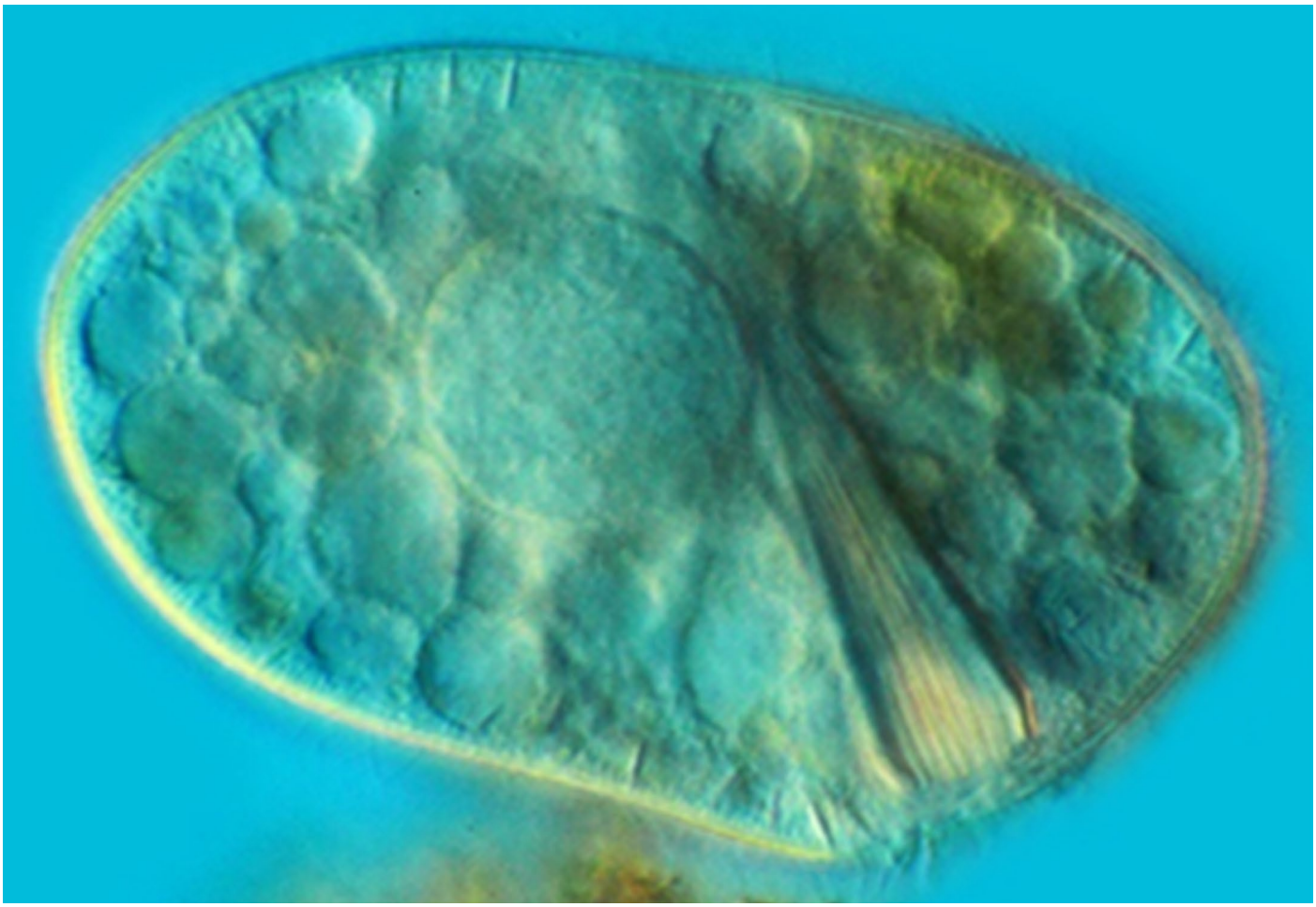

Fig 3. A micrograph of a ciliate of the genus *Nassula*, the class Nassophorea, and the family Nassulidae (WoRMS 2020be) featuring a prominent gullet lined with nematodesmata and a multitude of food vacuoles. Image courtesy of Antonio Guillén©, Proyecto Agua, https://www.Flikr.com/

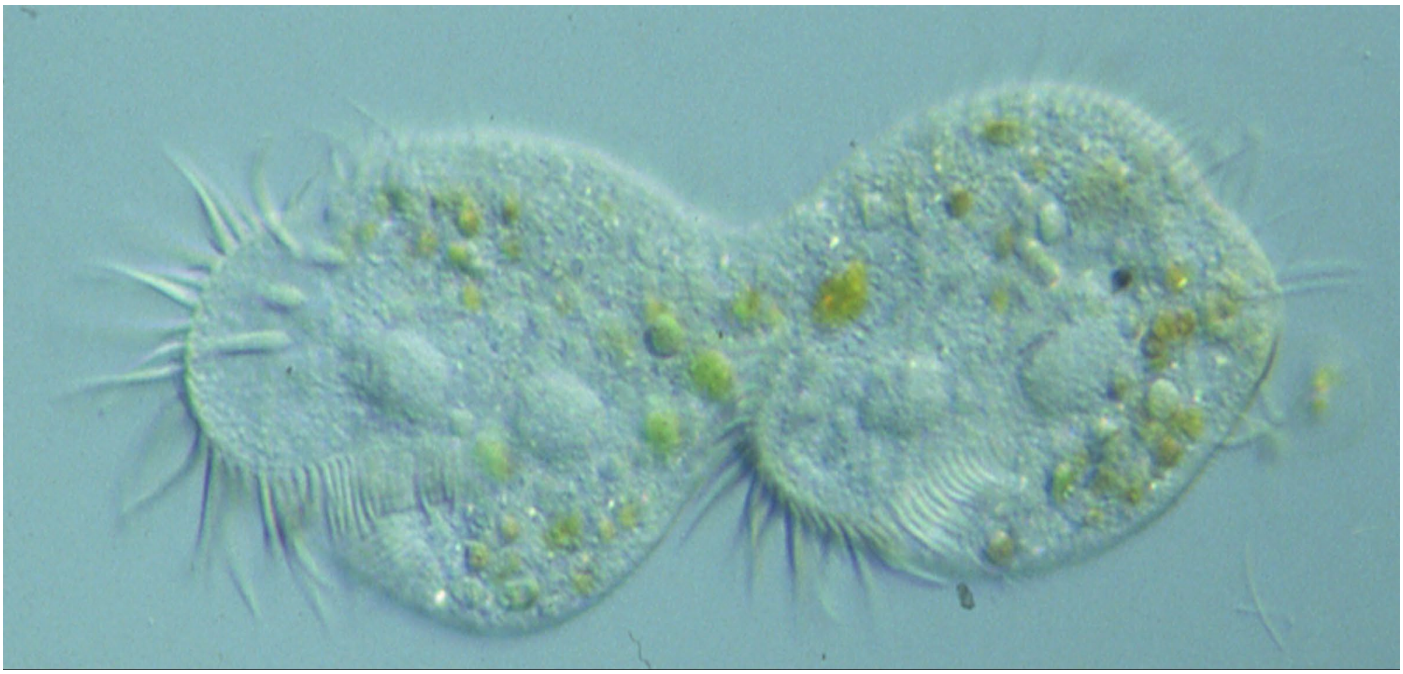

Fig 4. "Real-time" stomatogenesis of the proter from the opisthe in a non-*Cryptocaryon* ciliate with conspicuous oral ciliature that comprises polykinetids.

Divergent versus Convergent Evolution

Excluding epigenetic modification and phenotypic plasticity, eukaryotic evolution occurs over thousands of generations. Hence nominal rates of genetic mutation, Mendelian inheritance, and natural selection create divergence that develops a new species, or unrelated species evolve convergently to fill similar niches with comparable life cycles and phenotypes.

Fig 5. A quartet of regal tangs (*Paracanthurus hepatus*) "peppered" with marine "white spot" (cryptocaryonosis) caused by *Cryptocaryon irritans*. PhotoByMark ©, https://www.Flickr.com/

Cryptocaryon irritans is a unicellular ciliated chromist which causes marine "white spot", cryptocaryonosis, or marine "*Ich*" which is derived from the name of its freshwater counterpart, *Ichthyophthirius multifiliis*. Despite a remarkable similarity between the life cycles, cells, and diseases instigated by saltwater *C. irritans* and freshwater *I. multifiliis*, these organisms do not share ancestral lineage insofar as they arose from convergent evolution (Diggles & Adlard 1995; Colorni & Burgess 1997).

Taxonomy

As a member of the phylum Ciliophora, *Cryptocaryon irritans* Brown 1951 was reclassified in 2002 within the class Prostomatea, the order Prorodontida, and the nascent family Cryptocaryonidae (Wright & Colorni 2002; WoRMS 2022f). The World Register of Marine Species (WoRMS) does not acknowledge Cryptocaryonidae and positions *C. irritans* firmly within the family Holophryidae, whose strains are consequently ranked within the kingdom Chromista, the subkingdom Harosa, the infrakingdom Alveolata, the subphylum Intramacronucleata, and the infraphylum Ventrata (WoRMS 2022f).

As a former affiliate of the now obsolete subclass Holotrichia, *C. irritans* remains described as a holotrichous ciliate swathed in cilia of uniform length (Madoni & Ghetti 2004), whose theronts bear shorter oral cilia (Colorni & Diamant 1993; Colorni & Burgess 1997). *C. irritans* are conspicuous, economically significant ectoparasites of finfish kept in sea cages or recirculating mariculture systems (RMS). Notwithstanding, infections in wild teleosts are mild due to their short-lived free-swimming infective theronts (Fig 9. F) and their epithelium-integrated phoronts/trophonts (Fig 9. A & B; Colorni & Burgess 1997) which may be, albeit perhaps not (Cardoso et al. 2025), moderated by their regionally-acquired lasting protective immunity (Burgess & Matthews 1995). Aquarium- or sea cage-confined fish are exposed to a multitude of all stages (Colorni & Diamant 1993; Colorni & Burgess 1997), where a plethora of unsynchronised life cycles culminate in simultaneous infections and palliations. We explored the importance of osmoregulation and the ionic consistency of a fish's blood, cells, and viscera; however, *C. irritans* instigate perforations of the skin, eye, and gills which predisposes the afflicted to multiple organ failure.

Clinical Signs

Fish manifest whitish cream spots that fade during "daylight", insomuch as putatively photophobic phoronts/trophonts burrow deeper into the skin which elicits irritation, flashing, fin agitation, and sudden darting. Rapid gill irrigation indicates acute respiratory incursions, whereas moribund fish become listless and dehydrated (Bartelme 2003a). Healthy brachial filaments (primary gill lamellae) are defined and cherry red, whilst those of the infested will be pale and congested and aggregated with mucus (Fig 17.; Colorni & Burgess 1997).

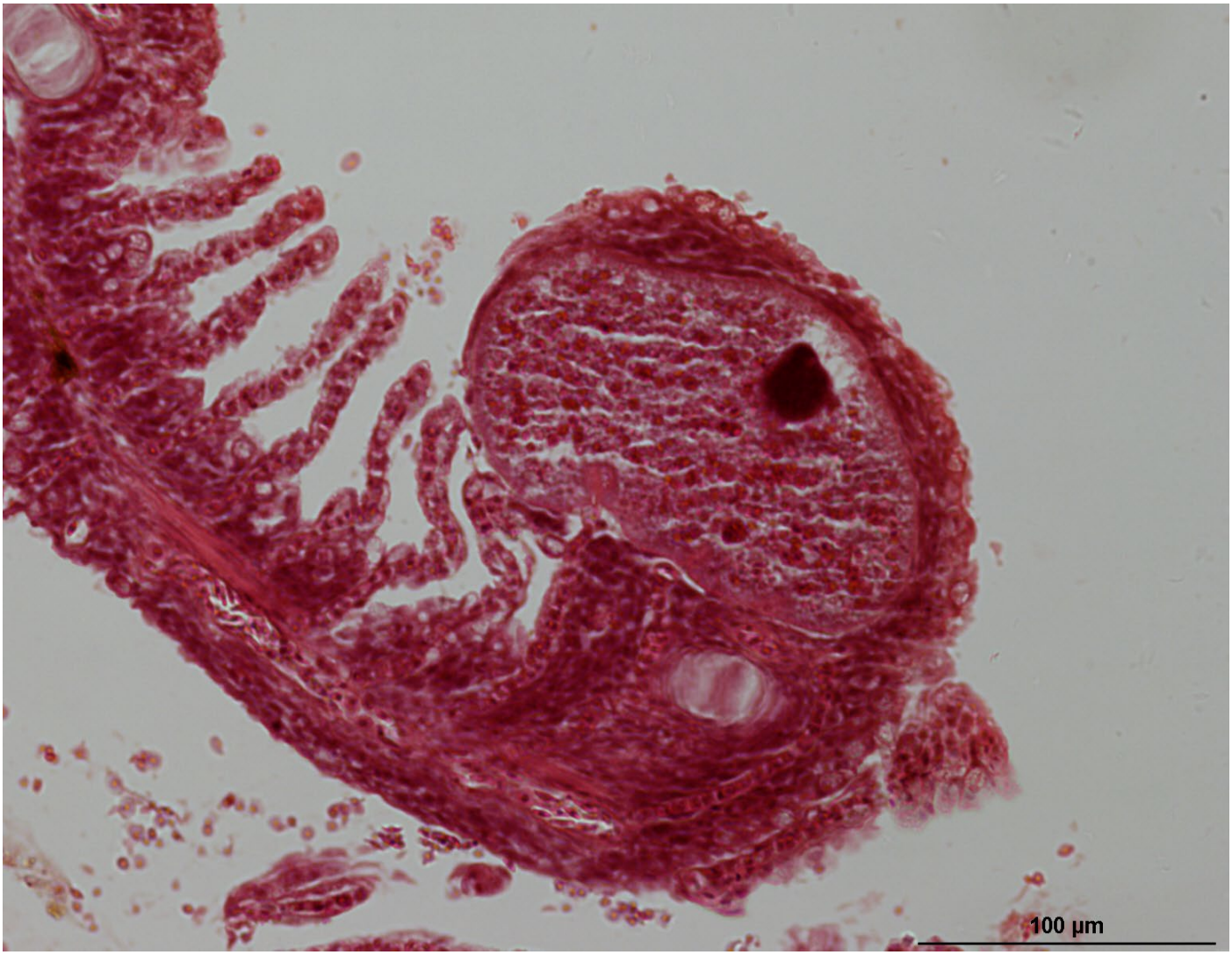

Fig 6. A remarkable micrograph of a phoront of the freshwater *C. irritans*-analogue, *Ichthyophthirius multifiliis* which has provoked fusion of the secondary lamellae and hyperplasia in the gill of the three-spined stickleback (*Gasterosteus aculeatus*). Contains public sector information licensed under the Open Government Licence (OGL) v3.0. Consult bibliography.

Life Cycles

Strains of *C. irritans* differ greatly in size, developmental timing, virulence, host range, and permissive temperature and salinity, and further disparities occur when dissimilar hosts are infected. It is impossible therefore to predict the duration of each life cycle (Yoshinaga 2001; Dickerson 2006). Accounts of days to weeks are not uncommon, although in captivity at tropical temperatures (23 to 27°C), each life cycle concludes within days and almost certainly within two weeks. Increasing the temperature of a fish-only system to 30°C enhances the efficacy of chemotherapeutics, and for fish and systems able to tolerate such a temperature, 34°C severely impairs trophont and tomont maturation which is redolent of a quasiviable therapy (Fig 9. B & D; Yoshinaga 2001). Corals cannot withstand such heat, whilst tropical marine fish appear unaffected at 30°C. Teleosts conserved at temperatures to which they are unsuited will stop feeding and may die, whilst exceeding their natural range may alter blood pH leading to stress (Bartelme 2004a). Chloride cells regulate the pH of teleostean serum (Cameron 1978), whereas the author suggests fish systems are not run at temperatures exceeding 30°C.

Theronts encapsulated in a chemotherapeutic-impenetrable cyst spiral in the water whilst they actively seek a host (Fig 9. F) where chemotaxis cannot be ruled out (Diggles & Lester 1996), insofar as the theronts of *Ichthyophthirius multifiliis* are attracted to various teleostean waterborne proteins (Buchmann & Nielsen 1999).

Theronts ordinarily develop from emerging tomites at night (Fig 9. E) and remain viable for merely 18 hours which exhibit positive phototaxis, which may predispose fish to infections at dawn, whereas contagion also occurs nocturnally. Several mature trophonts are shed from fish during darkness (Nigrelli & Ruggieri 1966; Burgess & Matthews 1994; Burgess & Matthews 1995; Colorni & Burgess 1997), whereas fish may acquire some degree of immunity (Burgess & Matthews 1995), but only to strains with which they are familiar (Wilson, personal communication). *C. irritans* and bacteria stimulate comparable immune responses (Khoo et al. 2012), where erythrocytes were scarce and "white cells" were enriched at 10 days post-infection which is symptomatic of anaemia and elevated humoral defence (Rigos et al. 2013).

Theronts burrow into epithelium within minutes and transform into phoronts that rapidly mature into trophonts (Fig 9. A & B) where they revolve impelled by their cilia (Roberts 1989) as they ingest whole cells and their debris (Colorni & Burgess 1997; Mo et al. 2016). Epithelial encapsulation protects them from predators, chemotherapeutics (Yanong 2009), and host immunity (Cardoso et al. 2025).

Fig 7. Black mollies (*Poecilia sphenops*) typically inhabit fresh and brackish water, yet they acclimate to full-strength seawater where they are widely used in research.

Somatic traumas arising from incursions of *C. irritans* are healed within 24 hours, while it only takes 60 minutes for theronts/phoronts to be enveloped in nascent gill epithelium (Fig 6.). As the trophont grows it swells and protrudes outwards which becomes evident as a whitish cream nodule, where several may occupy the same burrow such as those of the rays of fins. However, recurrent frenetic infections fuse secondary gill lamellae, and significant corneal incursions instigate hyperplastic opacity (Colorni & Burgess 1997).

Trophonts leave the fish typically in the early morning before sunrise or if the host dies, where their surface ridges flatten, their cilia are shed, and they descend as settling protomonts that crawl on substrate (Fig 9. C; Brown 1963; Burgess & Matthews 1994; Colorni & Burgess 1997). The other life phases are protected by an outer cyst or they are shielded within skin, and thus only protomonts and nascent tomonts are sensitive to chemotherapeutics: the former for merely 18 hours and the latter before encystment which takes up to 12 (Colorni 1985; Colorni 1992; Yanong 2009). Once encysted, dormant tomonts are capable of reactivation and tomite development after three months at 12°C (Zhao et al. 2023). An indefinite time elapses before the fruition of tomite daughters within anchored tomont cysts (Fig 9. D & E), where such rhythmic disparities ensure that life cycles remain unsynchronised and virulent.

Between 100 to 1000 tomites are spawned within tomont cysts that ultimately develop a germinal pore (Cardoso et al. 2025) through which their emanating contents quickly develop into infective encapsulated theronts (Nigrelli & Ruggieri 1966; Colorni 1985; Burgess & Matthews 1995; Colorni & Burgess 1997; Mo et al. 2016), where swarms were liberated between 02:00 and 09:00 throughout experimental challenges of saltwater-acclimated mollies (Fig 7.; Yoshinaga & Dickerson 1994). Theronts exhibit limited upward mobility of around 5 cm (2”; How et al. 2015), and although fish enter substratum-proximate nocturnal torpors, infectivity falls to less than 4 percent after six hours (Yoshinaga & Dickerson 1994).

In precis: theronts locate a host and burrow into their skin where phoronts transform into feeding and rotating trophonts. Satiated protomonts leave the host and descend to substrate where they crawl until encapsulated and adhered within tomonts, which are life stages susceptible to chemotherapeutics before tomont encystment at approximately 30 hours. Internal proliferation creates tomite daughters that ultimately mature into encapsulated theronts, where timing discrepancies throughout tomite division and development ensure life cycles remain asynchronous. Pore formation in tomont cysts liberates swarms of free-swimming tomites that transmute into host-seeking theronts (Fig 9.).

Variable Strains

Aetiology and virulence are determined by strain (Kaige & Miyazaki 1985), since an acutely lethal isolate from the Mediterranean previously unrecognised in temperate waters, caused mass mortalities within three days when it targeted the gills and tunnelled pervasively beneath the skin (Diamant et al. 1991). Colorni and Diamant used scanning electron microscopy (SEM) to study the stage transformations of a Red Sea strain (Colorni & Diamant 1993). The gestation of each metamorphosis was influenced by temperature and host, insofar as the timekeeping of tomite maturation was commensurate with each trophont-nurturing kind of teleost (Diggles & Lester 1996a). Host-attuned developmental intervals explain multiple unsynchronised life cycles within recirculating systems housing a variety of fish.

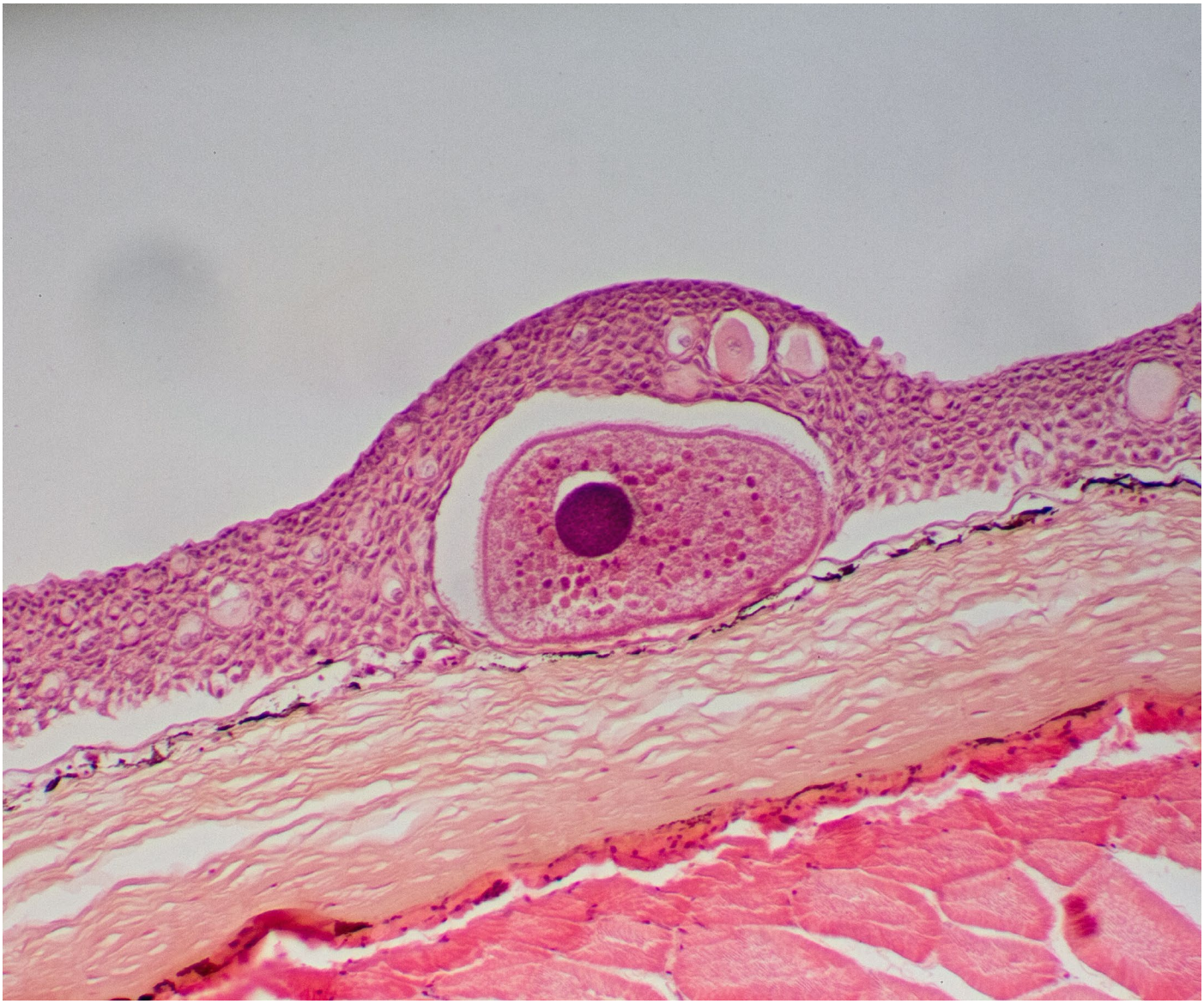

Fig 8. An epithelium-encapsulated freshwater trophont.

Jee and collaborators isolated *C. irritans*-like ciliates that achieved tomite division at 16°C and caused lamellae fusion and mucosal aggregations in the gills of Japanese flounder (*Paralichthys olivaceus*). Their tomonts divided into daughter cysts before their tomites developed, and all stages were larger than previously described (Jee et al. 2002). Hypo- and hyper-saline-resistant strains arise (Yambot et al. 2003) which foils previously proposed therapeutic approaches exploiting hyposalinity (Colorni 1985; Delbeek & Sprung 2005). Strains are prone to aetiological-inconsistencies like those that vary in tomont stickiness and clustering, rates of genetic mutation, pathologies of skin and gill, and those that withstand from 5 to 10 parts per thousand (‰; Yambot et al. 2003).

Host/Parasite Interactions, Virulence, and Fish Populations

Ornamental marine fish originate from worldwide tropical regions, where strains of *C. irritans* are geographically and regionally distinct (Diggles & Lester 1996b). *Siganus canaliculatus* rabbitfish moderate cryptocaryonosis (Fig 10.), because trophonts left the host prematurely, and protomont survival was curtailed, while those that endured developed into runt tomonts (Jiang et al. 2018).

Pathogens do not intend to cause mortality insofar as death of their host results in parasite demise, therefore host/parasite interactions attain a form of equilibrium, at which point, pathogenesis instigates merely moderate discomfort and malaise. Like human infections of influenza, co-existence becomes the objective in time; because Spanish "flu" diseased five hundred million, and forty million died in 1918 (Oxford 2000; Kilbourne 2006). Today it is rare for influenza to kill, yet it is predisposed to cause mortality in the elderly and young, because the virus is now in balance with its host (Penczykowski et al. 2015; Roser 2020). Exposing fish to a strain of *C. irritans* that they have never encountered often proves fatal, thus disease control ***is an absolute requirement*** in a commercial fish-only environment.

Parasite Attenuation

Overwhelming evidence and experience attest we must eradicate this pathogen in commercial systems. Retailers must not supply infected fish to reef aquaria, because the only effective remedy is copper sulphate which is lethal to invertebrates and ***must never*** be used in reefs.

A parasitic rampage results in a total loss of fish when a virulent strain of *C. irritans* enters a reef, whereas corals tend not to thrive in teleost-free systems. Survivors often sustain subclinical infections at the gill; hence aquarists consider these parasites

extinct. Hitherto infected systems must therefore remain fallow for well over six weeks before introducing a fish (Bartelme 2003), and safely maturing a reef populated with corals is a tricky business.

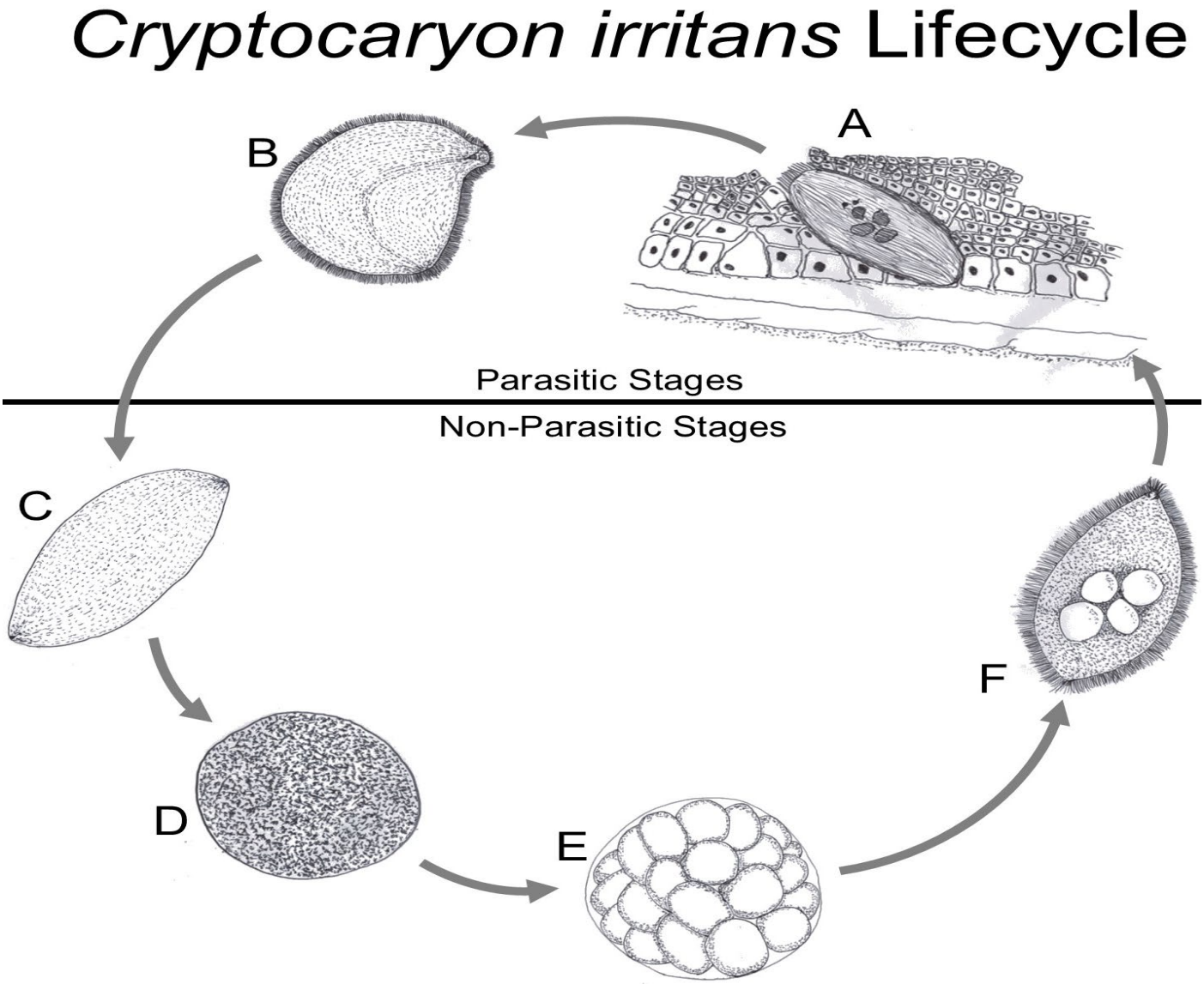

Fig 9. The life cycle of *Cryptocaryon irritans* which cause cryptocaryonosis: [A] phoront; [B] trophont; [C] protomont; [D] tomont; [E] tomites; [F] theront. Drawings of the accurate representations of biological structures within Colorni & Diamant 1993, Colorni & Burgess 1997, and Chi et al. 2020.

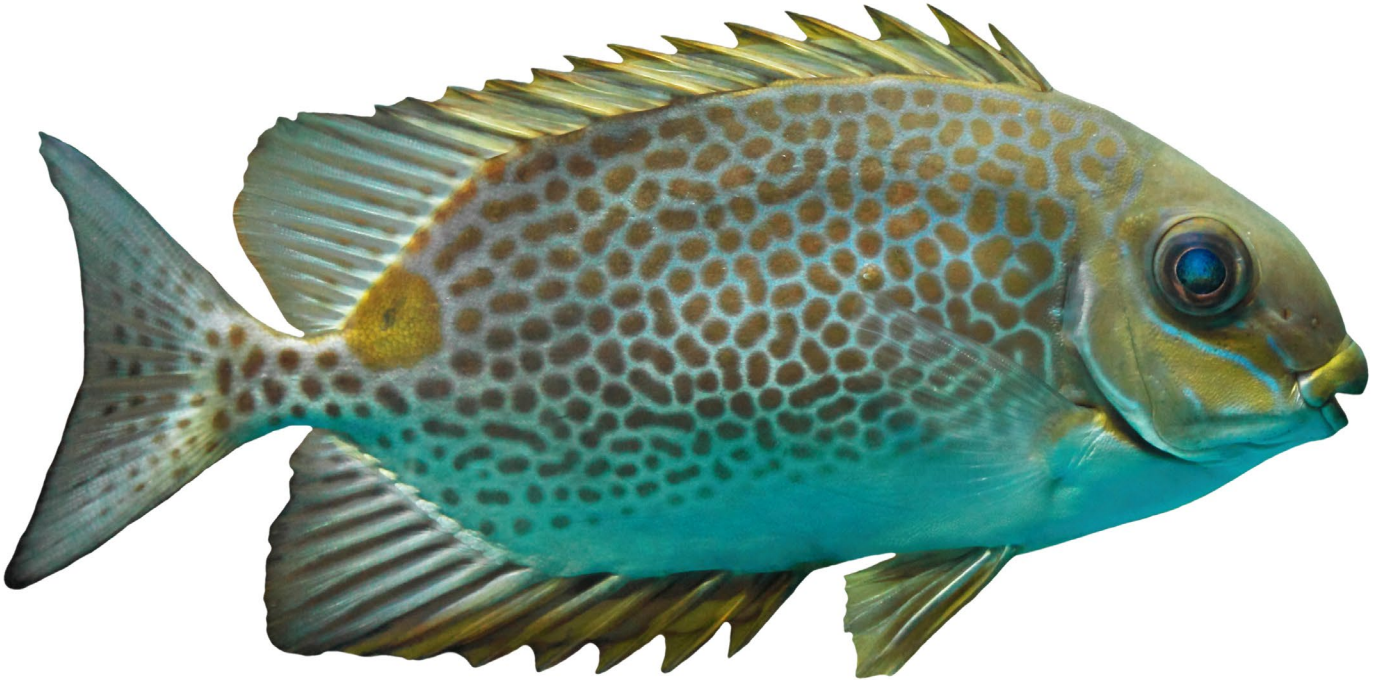

Fig 10. The rabbitfish, *Siganus canaliculatus* is somewhat resistant to marine “white spot”.

Fig 11. A regal tang (*Paracanthurus hepatus*) with a prominent scalpel presenting with the subtle yet clinical signs of marine "*Ich*", which may be carriers of temperate strains.

An Alleged Carrier

Regal tang (*Paracanthurus hepatus*) routinely breakout in moderate numbers of white spots which may be reservoirs of temperate strains (Fig 11.). Weber isolated a *C. irritans* tomont from a skin scrape of a regal tang which infers the entire life cycle happens on the fish (Weber 2013), because tomonts normally become affixed to substrate (Colorni 1985). However, incidental tomont adherence cannot be ruled out.

It is not uncommon for regal tangs to manifest clinical signs when other fish appear unaffected. Whether these occurrences are due to some unrecognised stressor is yet to be determined and perhaps future work can investigate these phenomena. An irritation-mitigating cleaner shrimp is advised when housing this quirky reef janitor, which will also benefit other fish; however, like all shrimp, they make for destructive food pilferers of fleshy Cnidaria (Fig 12.). They cannot extract trophonts, and despite the benefit of those assuming de-parasitising duties, cleaner fish become infected (Hedrick & McDowell 1995; Bartelme 2004a; Duffus et al. 2015).

The **Transcriptome** is the sum of all the messenger ribonucleic acid (mRNA) expressed from an organism's active genes that encodes for polypeptides that ultimately become functional proteins (Lexico 2019).

The "disease is always present" debate: myth or reality

The author is not declaring that disease cannot be eradicated in *fish-only systems*. Most can, but science has not discovered a way of eradicating viruses that infect fish, where many become carriers (Ciulli et al. 2015; Valverde et al. 2016).

As the author understands it, Bartelme appears to dispute that fish can carry illness or become reservoir hosts for pathogens in their 2003 article for Advanced Aquarist, while other researchers seem to concur (Pro 2012; Ho 2013). Bartelme may have focussed on the presence or absence of disease, and the author agrees that a virulent strain might kill the majority if not all aquarium fish. Nevertheless, a single life cycle of noncontagious and mild cryptocaryonosis seems to recrudesce on regal tangs, while countless numbers of the author's customers have noticed the same thing. A definitive diagnosis is made by excising a fin ray and microscopically observing the trophont revolving (Yanong 2009), where reports of this analysis are lacking perhaps due to the challenges of stress-free capture in a reef. All the same, excising a fin ray without anaesthetic will cause discomfort and distress, whereas transcriptome analyses of *C. irritans* revealed unique genes that proved useful as diagnostic biomarkers (Lokanathan et al. 2010).

Phylogeny facilitates elucidation of ancestral lineage according to the genetic distances of identical or similar genes called homologues, where genotype, phenotypic traits, origin, and geographic location often disclose an organism's inclination to act as a reservoir host (Cronin et al. 2010).

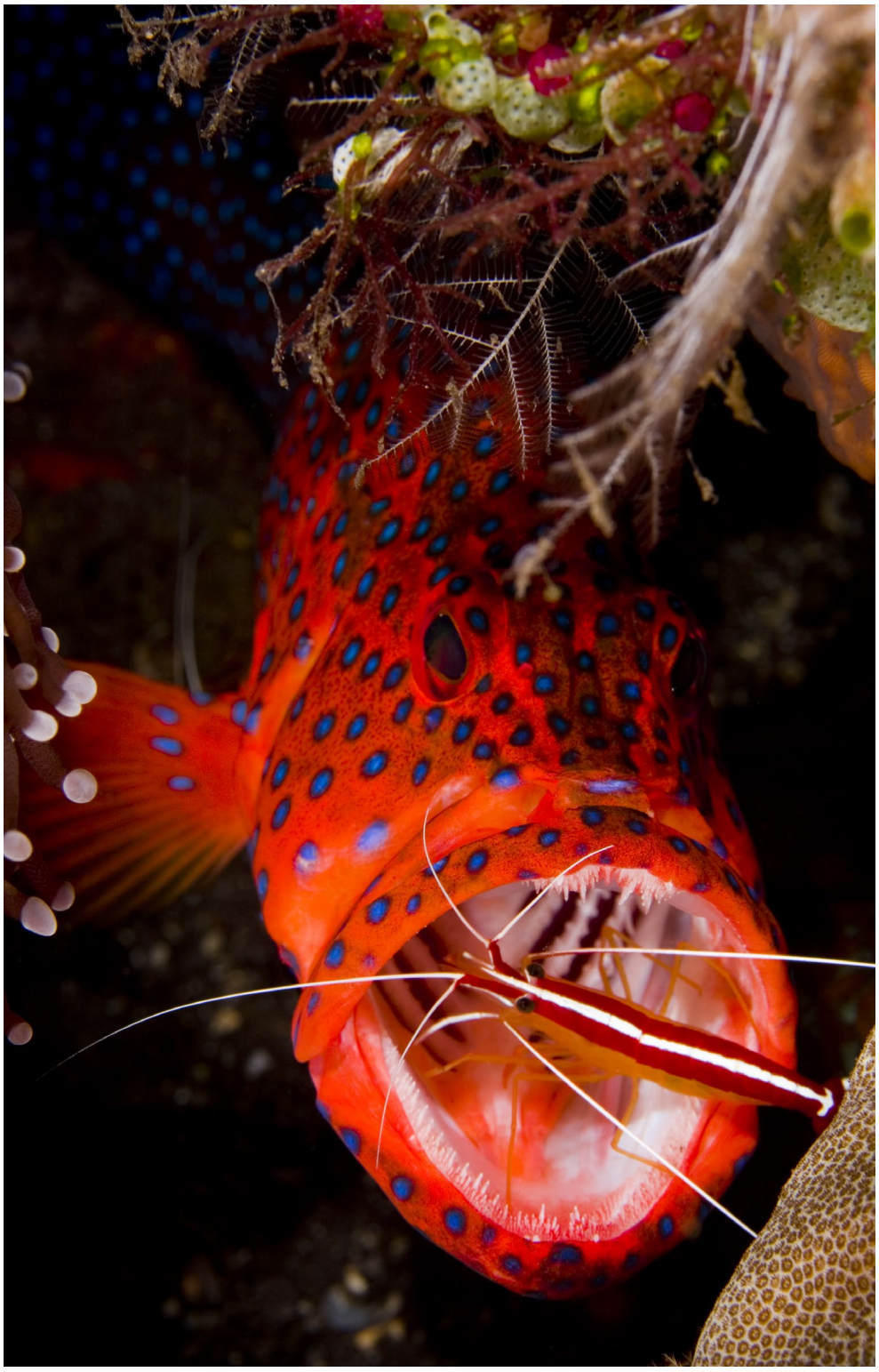

Fig 12. A far more valuable than the next meal cleaner shrimp (*Lysmata amboinensis*), cleansing the mouth of a coral grouper (*Plectropomus leopardus*).

The parts of a fin that adjoin the rays do not carry nerves whereas rays are exceptionally perceptive. Numerous academics have suggested that fish do not feel pain like humans. Although contentious and beyond the scope of this article, the author has kept fish for over 55 years and the suggestion that they have a lower capacity to feel pain and anxiety appears erroneous. Rose and collaborators concluded that fish do not experience discomfort like us, yet Hardy and researchers discovered that the sensitivity of a dorsal fin is comparable to that of our fingertips (Rose et al. 2014; Hardy et al. 2016).

A clean incision made between the rays of a fin painlessly and temporarily marks a fish post-processing, which proves helpful following administration of therapeutics or vaccines.

Nature continues to inform us that disease is omnipresent. Contracting the chickenpox virus (varicella-zoster virus; VZV) twice increases the likelihood of presenting with shingles in later life (Croen & Straus 1991) and *Toxoplasma gondii*, the chromists that cause toxoplasmosis in immunocompromised humans, are contracted from cat scratches where the initial infection is subclinical. Obligate intracellular parasites that cannot survive long outside their host (Kochanowsky & Koshy 2018), death by toxoplasmosis in immunocompromised cat owners is not unusual (Lindsay et al. 1997).

The chromists *Plasmodium vivax* infect red blood cells which cause malaria in human carriers (Howes et al. 2015), while our gut nurtures innocuous strains of *Escherichia coli* and species of *Clostridium* and *Salmonella* (Todar 2012). Furthermore, a consortium of microorganisms constitutes the microbiome that is human skin (Grice et al. 2009; Hannigan et al. 2015).

We discussed how virulence varies amongst strains, and we comprehend the power of immunity. Sometimes defence forces a pathogen into dormancy as opposed to being purged, which induces a carrier state (Croen & Straus 1991; Kochanowsky & Koshy 2018).

Fish subjected to acute or chronic stress become immunocompromised (Tomasso 1994), albeit a compromised immune system is not a prerequisite for infection, immunocompromised fish, like corals and us, can become vulnerable to illnesses that they would ordinarily withstand (Price 1985; Bartelme 2003). The mechanisms enforcing dormancy are annulled if the immune system is weakened, which results in disease recrudescence, and it is counterintuitive to suggest that once a pathogen is actively multiplying within a recirculating system, other fish will not be vulnerable. Remarkably so, if the stressor acting on the reservoir was operative in them. Nevertheless, expectations remain the death of objectivity.

Coral holobionts include viruses, bacteriophages, fungi, chromists, protozoa, and bacteria (Clerissi et al. 2018), and thus why would the gut, skin, internal or external organs of fish be different.

Fig 13. Left - a copperband butterflyfish (*Chelmon rostratus*). Bottom right - a moorish idol (*Zanclus cornutus*). These otherwise healthy examples appear infected.

Notwithstanding the anecdotes of legions of aquarists and the discovery of a skin-adhered tomont on an example of *Paracanthurus hepatus*, we lack corroborating evidence for the completion of the *C. irritans*' life cycle upon a fish (Weber 2013). Nevertheless, asymptomatic carriers of the fish pathogen *Amyloodinium ocellatum* harbour gill tomonts (Kuperman & Matey 2000; Seoud et al. 2017) and *Lymphocystis* virions and their nucleic acids are routinely identified in asymptomatic teleosts (Cano et al. 2009; Ciulli et al. 2015; Valverde et al. 2016; Valverde et al. 2017; Leiva-Rebollo et al. 2019). Antibiotics that attenuate the pathology instigated by *Mycobacterium marinum*, drive the bacteria into latency which are carried (Colorni et al. 1998, cited in Colorni & Diamant 2014). Fish become reservoir vectors of *Ranavirus* that are transmitted to vertebrates that share water (Mao et al. 1999; Robert & Jancovich 2016), whilst rainbow trout carry virions with no clinical signs (Whittington & Reddacliff 1995). The *Herpesvirus* endemic in catfish populations, channel catfish virus (CCV), is vertically transmitted from mother to offspring and a subpopulation remain carriers (Thompson et al. 2005). Moreover, *Photobacterium damsela* subspecies *damsela* are zoonotically fatal to humans and fish, whose dormant carrier phase commences below 21°C (Magariños et al. 2001; Toranzo et al. 2005; Colorni & Diamant 2014).

Disease in a population of chronically- or acutely-stressed fish, looks like a single pathogenic contagion, particularly so, in the same species. Different kinds of teleost vary in their susceptibility to a multitude of illnesses, and likewise, resistance differs amongst conspecifics (Price 1985). Nevertheless, stress is epitomised by the simultaneous manifestation of distinct illnesses in a variety of species.

Administration of a "suppressing fire" of chemotherapy prevents the spread of communicable disease in commercial fish-only systems. Precise dispensation ensures the fish remain unharmed and benefit greatly, but this type of prophylaxis cannot be used in reefs. The immune system remains an organism's best defence, and strategies to boost immunity combined with rigorous maintenance, optimal system design, and exceptional husbandry are ordinarily sufficient.

The Chain of Supply

As responsible aquarists are efforts are best focused on encouraging the *proper* use of chemotherapeutics and supporting retailers that get it right. Presently, the husbandry of leading livestock suppliers is safeguarded for fear of scandalous slander by the inexperienced and ill-informed. It is unthinkable that a fish carrying an eye parasite or a virulent pathogen is purchased for a reef. It would be refreshing to hear outlets openly discuss control of communicable disease. The author did not shy away from these conversations at the Reef Ranch™. Misconceptions have driven these debates underground; the subject is taboo. Even eye parasites (*Neobenedenia melleni*) can be eliminated with a trilateral approach. All is revealed in Aslett 2024. However, until the "reefing" community concurs, copper-based remedies discolour translucent silicon blue, and black alternatives exist, but they curtail aquarium longevity. Remind your customers to never pollute their system's water with a supplier's, *any supplier's.*

Relevant Experience

Commercial fish-only recirculating systems operated without copper-based treatments run trouble-free for finite periods until a fish is introduced carrying a virulent pathogen, whereafter all livestock are exhibiting clinical signs within 48 hours. This is truly devastating for a business because the fish cannot be sold under any circumstances, the animals are jeopardised, and the business is likely to

Fig 14. A bannerfish (*Heniochus diphreutes*) manifesting the subtle signs of disease.

fail. Fish systems devoid of therapeutics have been infected with virulent strains of *C. irritans*, yet it is possible to eliminate the pathogen without losing a single fish by correctly applying a copper- and formalin-based remedy. However, certain precautions *must be* observed.

Fish, Aquaria, and Commercial Recirculating Systems

Housing fish in a commercial ornamental recirculating system without disease control jeopardises the livestock and the proprietor's income. *For several reasons, a commercial fish-only system will never be analogous to any form of aquarium, including large public displays*, insofar as correctly operating these systems requires expert knowledge, rigorous upkeep and practices. The key dissimilarities comprise a constant influx of livestock infected with exotic pathogens, which exert inordinate pressure on the biofilter consortium. Guarantee success by consulting: The Complete Reef Aquarist (Aslett 2024).

Peer-reviewed white literature proposes *C. irritans* is mitigated by cupric ion (Cu^{2+}) concentrations from 0.15 to 0.2 mg l^{-1} (ppm; Yanong 2009) which exceeds the dose tolerated by marine ornamental teleosts. Their therapeutic range extends from 0.075 to 0.1 mg l^{-1} (ppm). 0.1 mg l^{-1} is the uppermost limit which *must not be* exceeded, yet it is twice the concentration typically used for ambient control. Furthermore, fish *must be* gradually exposed to protracted increases, because abrupt rises in chemotherapeutics are responsible for the detachment of gill epithelia from their basement membranes which increases critical diffusion distances that lead to catastrophic asphyxiations (Figs 17. & 18.). All livestock may be lost, hence see below: Chemotherapeutics: critical information proceed with caution.

Copper Alternatives

The persistence and lethality of *C. irritans* has motivated aquarists and mariculturists to devise novel tactical and chemotherapeutic approaches, but only a few like hyposalinity and the transfer method have proven efficacious (Bartelme 2004). Corals and several elasmobranchs such as sharks and rays cannot withstand hyposalinity (Lowry 2004) which appear naturally resistant to *C. irritans* (Lom 1984, cited in Noga 2010a), whereas most strategies are impractical for commercial ventures. Immersion baths may be useful for intensive rearing facilities, whereas the most successful and innovative manoeuvre, exploited copper alloy sea cages (Yin et al. 2019). Pre-encapsulated tomonts were destroyed by exposing them to three hours of 10 $^{o}/_{oo}$ (ppt; 10 g l^{-1}) every three days (Colorni 1985).

Chemotherapeutics

Freshwater dips are ineffective and cause osmotic shock and immunosuppression, which is exacerbated in the profoundly punctured, whereas 40 $^{o}/_{oo}$ (40 g l^{-1}) hypersalinity proves significantly injurious (Colorni 1985; Bartelme 2004a). Lowry introduced ammonia-detoxified and temperature- and pH-adjusted plane-fresh imports into a SG of 1.008 (10 $^{o}/_{oo}$; Lowry 2004). No adverse effects were observed in a wide variety of teleosts where most were feeding voraciously the next day. *C. irritans* was eradicated, whilst attenuation of flatworms and other parasites became evident (Halton & Morris 1969; Lowry 2004).

Emperor angelfish (*Pomacanthus imperator*) effortlessly acclimate to 7 $^{o}/_{oo}$ (0.7 percent; Woo & Chung 1995). Numerous inshore reef-specialised species appear euryhaline compared to pelagic. Some survive 3 $^{o}/_{oo}$ but most favour 10 (Wu & Woo 1983; Bartelme 2004b). Research findings are redolent of more or less salty prehistoric seas (Spaargaren 1979; Sanford et al. 2013); and therefore, fish may have evolved to suit a range of salinities. If that be the case, why are corals stenohaline (Ailsa & Ross 2003; Kerswell & Jones 2003; Bartelme 2004b; Seveso et al. 2013; Hedouin et al. 2015), insofar as finfish emerged in the Devonian around three hundred and ninety-seven million years ago (MYA), whereas coral progenitors ascended one hundred and three million years earlier in the Ordovician (Burchett & Dando 1996).

Malarial Treatments for Humans

Antimalarials like quinacrine hydrochloride exhibit partial efficacy; however, fins deteriorated, and fish skin darkened which is indicative of stress (Bartelme 2004a). 100 mg kg^{-1} of body weight (BW) per day (d^{-1}) of 1 percent dietary quinine safely diminished trophonts on day three in gilt-head sea bream (*Sparus aurata*). No effect was observed in the medicated or control group by day five, whereas the diet was rejected on days 10 and 15. Despite its capacity to forestall mortalities and moderate trophonts, quinine showed limited merit, whereas fish erythrocytes remain more sensitive to the haemolysis caused by antimalarials (Rigos et al. 2013).

An archaic malarial treatment has been revered as a cure-all for dedicated fish systems, yet these kinds of drugs must *never be* used in reefs. Chloroquine has moderated *C. irritans*, *Aiptasia* species of nuisance anemone, parasitic dinoflagellates like *Amyloodinium ocellatum* which are the causative agents of marine velvet, and the necrotic ulceration-eliciting scuticociliates, *Uronema marinum* (Pérez-Uz 1996; Noga 2010; Hemdal 2013). However, peer-reviewed evidence corroborating its safety and

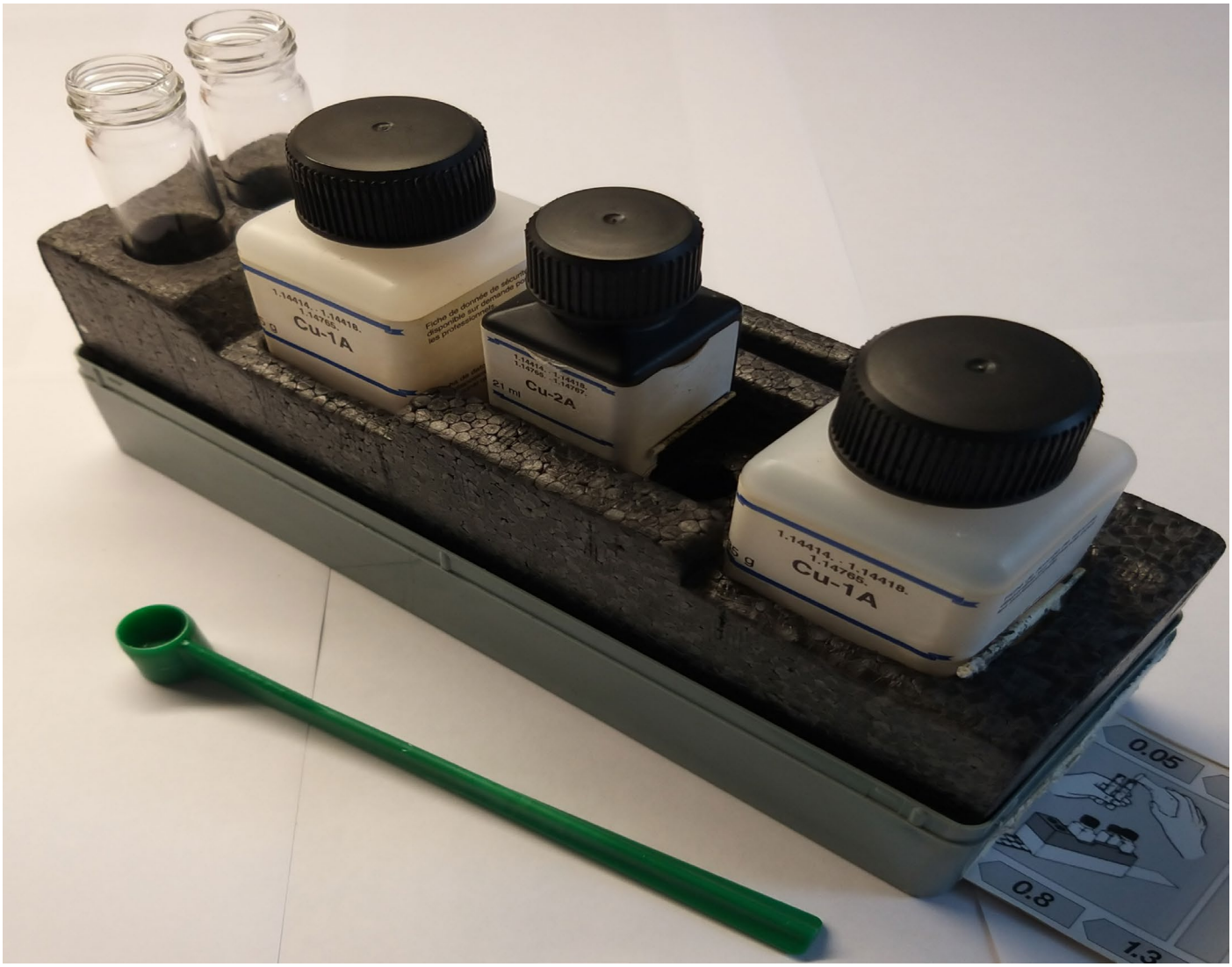

Fig 15. An ultraprecise analytical grade cupric ion (Cu^{2+}) assay for seawater (Merck product 1.14414).

Fig 16. Emperor angelfish (*Pomacanthus imperator*) are euryhaline insofar as they thrive at 7 ‰ (Wu & Woo 1983).

efficacy appears lacking, whilst chloroquine inhibits the activity of the vital immune signals liberated by human macrophages and monocytes: tumour necrosis factor alpha (TNF-α), interleukin 1beta (IL-1β), and interleukin 6 (IL-6; Jang et al. 2006). A chloroquine-enriched diet enhanced the autophagy and thus the survivorship of fathead minnows (*Pimephales promelas*) challenged with haemorrhagic septicaemia virus IVb (Liu & Lumsden 2020).

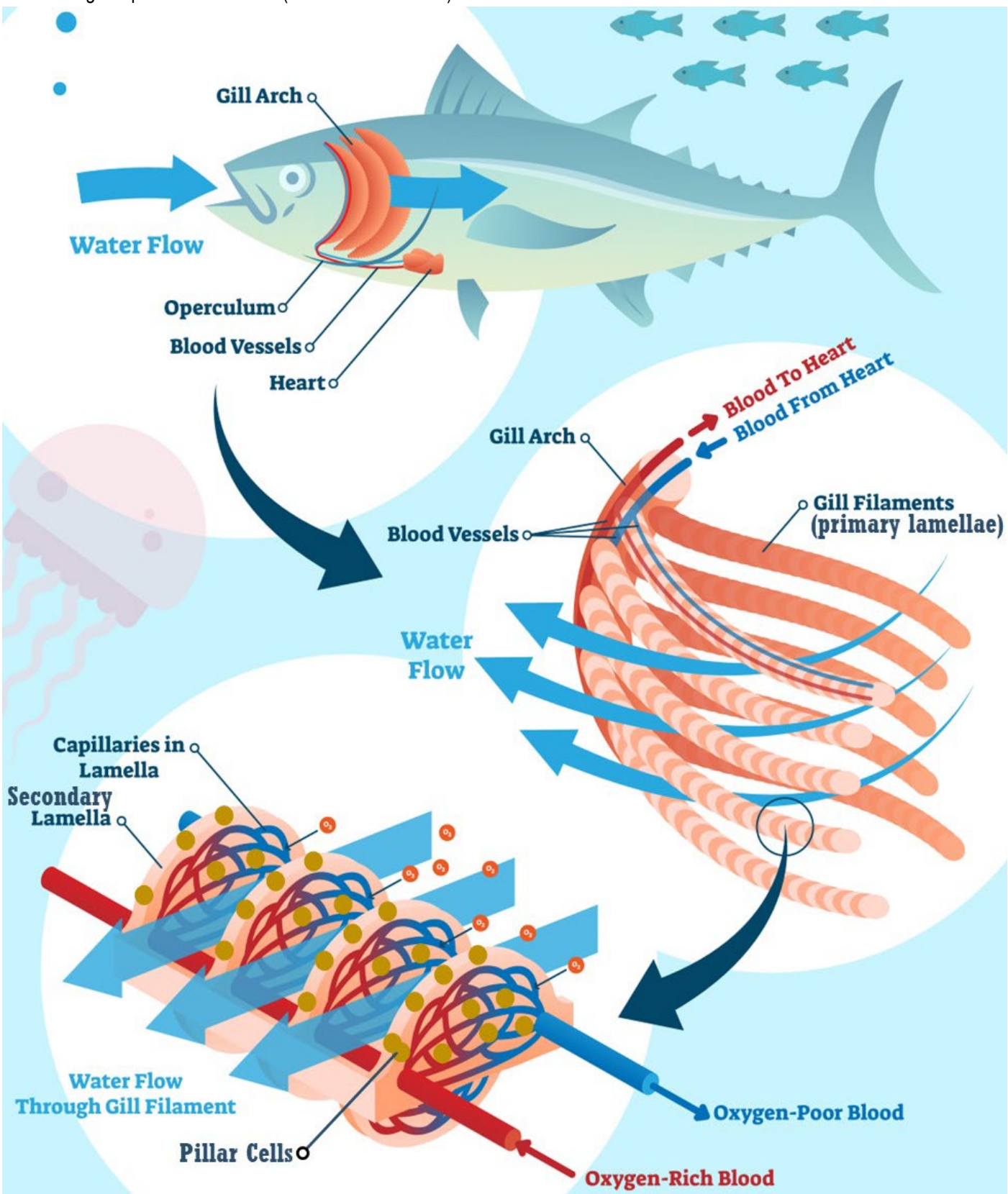

Fig 17. A diagram exemplifying the function of primary and secondary gill lamellae.

Medicinal chloroquine ranges from 10 to 20 mg l^{-1}, insofar as 10, 15, and 20 have been used for a quarantine prophylactic, a generalised antichromist, and to clear tenacious infestations of *U. marinum* (Noga 2010; Hemdal 2013). The most accessible form of chloroquine is chloroquine "diphosphate" (Aralen; $C_{18}H_{26}ClN_3.2H_3PO_4$) which comprises orthophosphoric acid. Wear personal protective equipment (PPE) because the powder becomes statically charged and sticks to utensils and paraphernalia, which must be pre-dissolved in water. Residual chloroquine may be ergonomically assayed using an ultraviolet (UV) spectrophotometer because its concentration is directly proportional to its absorption at 392 nanometres (nm; λ_{392}). Quartz glass cuvettes must be used in the

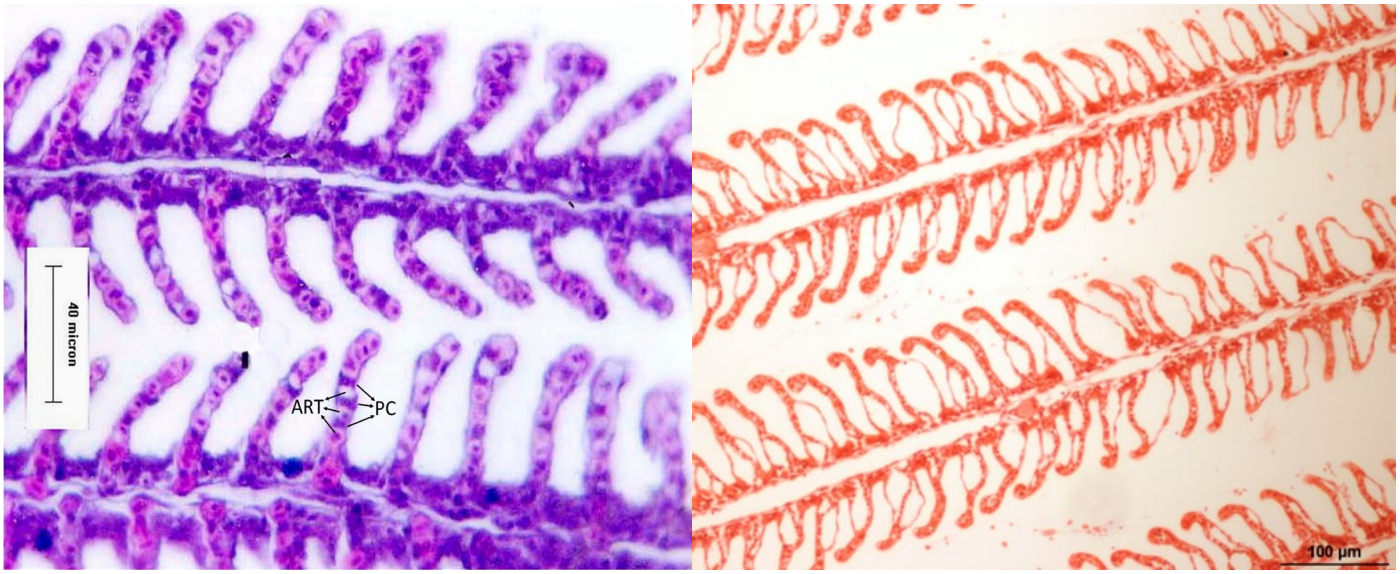

Fig 18. Left - a brightfield micrograph of a cross-section through the secondary lamellae of healthy fish gills featuring pillar cells [PC] and arterioles [ART]. Right - a similar micrograph of atypical embolised secondary gill lamellae from a deceased rainbow trout where epithelia have separated from their basement membranes. This fatal pathology was conceivably caused by a dosing accident with malachite green and formalin. Contains public sector information licensed under the Open Government Licence (OGL) v3.0. Consult bibliography.

spectrophotometer because ordinary glass absorbs UV, whereas granular activated carbon (GAC) generates interference which is withheld throughout therapy.

Standard solutions of 25, 20, 10, 5, and 2.5 mg l^{-1} (ppm) are generated by adding varying quantities of chloroquine to test tubes of system water, which are evaluated using percent absorption at 392 nm. The findings are then plotted on a graph of mg l^{-1} of chloroquine versus absorbance (Fig 19.). The best fit line thus facilitates rapid estimation of residual chloroquine (Hemdal 2013).

Chloroquine "diphosphate" comprises around 20 to 30 percent orthophosphate, which fuels blooms of nuisance algae in well-lit fish-only displays. "Diphosphate" alternatives include chloroquine hydrochloride (Aralen HCL; $C_{18}H_{26}ClN_3.2HCl$) and chloroquine sulphate (Plaquenil; $C_{18}H_{26}ClN_3.H_2SO_4$; Hemdal 2013) which effectively comprise hydrochloric and sulphuric acids which are likely to initiate pH instability and acute and chronic stress. In preference dose chloroquine "diphosphate" ($C_{18}H_{26}ClN_3.2H_3PO_4$) in subdued light and reinstate illumination after re-establishing phosphate-limitation using Kent Marine phosphate sponge. However, *never* purge phosphate from commercial fish-only systems that support algal blooms.

Domestic fish displays may be run indefinitely on ambient lighting sufficient to awaken animals before feeds, where sudden increases in irradiance startle, so enable daylight to permeate the room.

Intriguingly, 20 mg l^{-1} of hydroxychloroquine sulphate (Reuquinol® Aspen) cleared all the epithelium-encapsulated trophonts of *C. irritans* in eight aquarium-confined marine ornamental teleosts within three days. Furthermore, what appears to be an extraordinary efficacious antimalarial and cryptocaryonicide did not appear to negatively impact the health of the tangs: *Acanthurus achilles*, *A. nigricans*, *A. ocellaris*, *Zanclus cornutus,* and *Z. desjardinii,* the copperband butterflyfish *Chelmon rostratus*, the royal angelfish *Pygoplites diacanthus*, and the foxface rabbitfish *Siganus vulpinus*. Notwithstanding the publication of this report in Istanbul's journal of aquatic sciences and engineering, it is crucial we recognise that this miraculous evidence was acquired under non-experimental conditions. The researchers remarked that thorough and rigorous objective investigations were necessary to corroborate the efficacy, safety, and suitability of hydroxychloroquine sulphate (Cardoso et al. 2025). Hence, *the author does not endorse antimalarials*.

C. irritans Mitigation: The Transfer Method

Life cycles are interrupted when fish are moved from one aquarium to another every three days, whereas used aquaria are emptied, sterilised with hydrogen peroxide, and dried before refilling with freshly prepared saltwater (Colorni 1985). Similarly, some tomont cysts are eliminated by depositing fine substrate in aquaria and syphoning it out every three days (Colorni 1987).

Hyposalinity

30 days of 15 $^{o}/_{oo}$ (15 g l^{-1}; 1.011 SG) eradicates the tomonts of hyposaline-intolerant strains (Noga 2000; Yambot et al. 2003; Bartelme 2004; Yanong 2009) which is used exclusively in teleostean systems and prohibits sales (Lowry 2004). Maximum daily variations of less than 2.5 $^{o}/_{oo}$, protect the biofilter consortium (Noga 2000; Delbeek & Sprung 2005; Yanong 2009) where internal salt concentrations are ~10 $^{o}/_{oo}$ (10 g l^{-1}; 1.008 SG), where marine finfish can supplement their requirements by the accrual and scavenging of salt. Research asserts hyposalinity is well-tolerated by marine teleosts (Bartelme 2004; Soengas et al. 2019).

Cell Culture is the propagation of living eukaryotic cells within laboratory petri dishes.

Vaccinations

In vitro culture of theront-infected fish cell lines sustains life cycles without injuring living hosts (Yoshinaga et al. 2007). The recombinant ciliate, *Tetrahymena thermophila* was engineered to express a gene of the *C. irritans* surface protein, GDCI3 i-antigen (Collins & Gorovsky 2005; Mo et al. 2019). The purified molecule was used to vaccinate groupers, whose immunity raised *C. irritans*-specific antibody M (immunoglobulin M; IgM) which enhanced the survivorship of the experimentally challenged. This was the first account of an efficacious vaccine for cryptocaryonosis (Mo et al. 2019).

UV sterilisation may kill theronts but they must enter the steriliser which are typically liberated from mature tomont cysts adhered to the displays in the vicinity of sleeping hosts. Lethal doses of UV are in the region of 800000 µW-s cm^{-2} (µJ-s cm^{-2}; Colorni & Burgess 1997; Yanong 2009), whereas research suggests UV sterilisation is ineffectual at mitigating encysted parasites compared to downward percolation through deep, fine sand (Bomo et al. 2003).

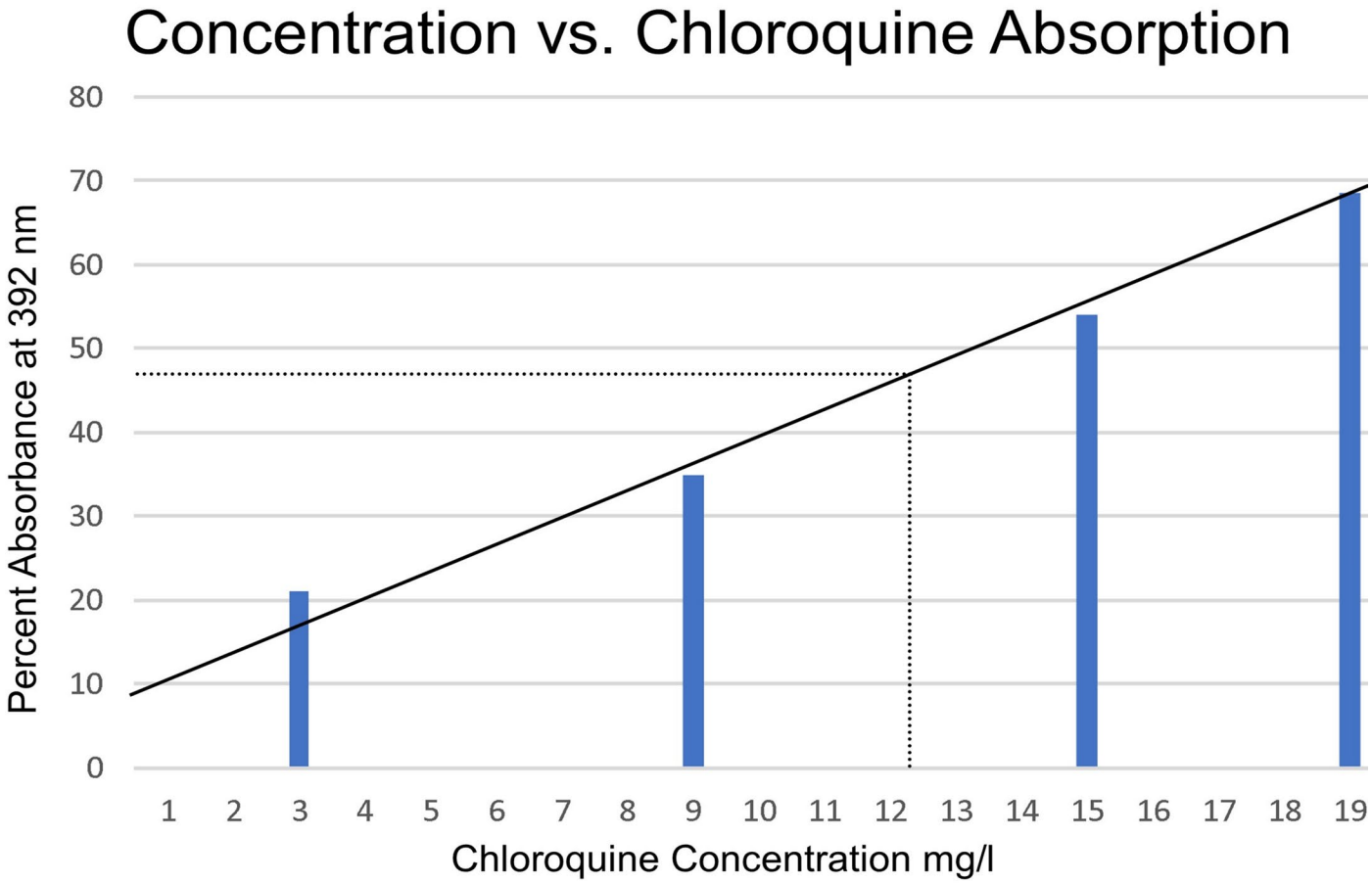

Fig 19. A graph of chloroquine concentration expressed in mg/l (mg l^{-1}) versus absorption at 392 nm whose best fit line quickly provides residual systemic chloroquine using a reading from an ultraviolet (UV) spectrophotometer. Adapted from Hemdal 2013.

Bomo and collaborators utilised passive downward seepage through deep columns of biologically active sand which removed 99.999 percent of bacteria (Bomo et al. 2003). Observations made under nonexperimental but real-life conditions, attest systems have been run copper-free with efficient and well-maintained UV sterilisers and pressurised sand filters, which does little to prevent an inevitable systemwide infestation. Hence mature pressurised sand filters appear no obstacle to theronts.

Chemical and Herbal Remedies

Octanoic (caprylic) acid is an eight-carbon saturated fatty acid (CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-C=O-OH) which attenuated the theronts of *C. irritans* when fed at a rate of 75 mg kg^{-1} BW d^{-1} yet failed to prevent mortality at 24°C (Hirazawa et al. 2001). Experiments performed on the freshwater chromists, *Ichthyophthirius multifiliis* established dissolved allicin and sodium percarbonate lessened theronts. The former is the juice squeezed from a clove of garlic (*Allium sativum*) and the latter is a chemical that releases hydrogen peroxide. 117 to 570 mg l^{-1} of garlic extract may kill tomonts (Buchmann et al. 2003), whereas biweekly exposure of rainbow trout (*Oncorhynchus mykiss*) to 50 and 100 mg l^{-1} of sodium percarbonate proved nondetrimental (Bartelme 2004). Hydrogen peroxide (H_2O_2) is a destructive bleach and reactive oxygen species (ROS), while the sensitive parts of fish such as the gills are exceedingly vulnerable, and hardiness varies interspecifically. Neither hydrogen peroxide nor sodium percarbonate are therefore sanctioned here. 5 µl ml^{-1} of allicin immobilised theronts and protomonts, whilst infected fish fed 20 percent garlic powder experienced caudal fin remission, yet it proved ineffectual at ameliorating parasitisations of the gill (Kim et al. 2019). Remarkably, tissue-bound allicin may camouflage the host from theronts (Cortes-Jorge 2000).

76 mg l^{-1} of aqueous green tea extract (AGTE) killed 95, 52.8, and 100 percent of theronts, protomonts, and tomonts within 12 hours by disrupting the parasites osmoregulation and mitochondrial integrity. The most potent catechin in AGTE, epigallocatechin (EGC) was tomonticidal at 40 mg l^{-1} after 12 hours, whereas double the concentration of gallic acid (GA) was needed to attain an equivalent efficacy (Yuan et al. 2025). One hour of 48 mg l^{-1} of a parasitic cellular membrane-perforating viscosin-like lipopeptide surfactant called biokos, which is synthesised by bacteria of the genus *Pseudomonas*, killed most theronts and protomonts in vitro, whereas 95 percent remission occurred in experimentally-challenged seawater-acclimated black mollies (Watanabe et al. 2023).

Honokiol is one of several bioactive compounds extracted from the bark of *Magnolia officinalis* which induces preprogrammed cell death (apoptosis) in cancers, the yeast-like microorganisms that cause human thrush (*Candida albicans*), and the tomonts of *C. irritans* after four hours at 1 mg l^{-1}. Honokiol appears to induce tomont apoptosis by inhibiting endoplasmic reticulum (ER) protein 44 (ERP44) which typically regulates the abundance of calcium ions (Ca^{2+}). Ionic accrual therefore elicits the caspase cascade whereby cysteine proteases cleave numerous key components which culminates in cellular disassembly (Abeomics 2025). See: Aslett 2024a's

figure 7. Nevertheless, only around 53 percent of tomonts died (Zhao et al. 2023).

Probiotics

Environmentally-enriched *Bacillus licheniformis* responded to *C. irritans* by differentially expressing thousands of their genes, which interrupted the development of theronts from tomonts by meddling with their fundamental intracellular processes (Jiao et al. 2023).

Chemotherapeutics: critical information proceed with caution

As with all potentially hazardous techniques, the author, websites hosting this article, and Reef Ranch Publishing Ltd assume no responsibility for injury, financial loss, or harm to persons, pets, possessions, or livestock that results from adopting the practices described herein.

Copper-based remedies are toxic to all invertebrates including corals, whereas lionfish, pufferfish, dwarf angelfish, mandarinfish, and cartilaginous fish of the parvphylum Chondrichthyes like chimeras, rays, sharks, and skates are more sensitive to copper, for which these therapies may prove unsuitable (Bartelme 2004). However, such remedies have been used to clear their infections (Thoney 1990) whilst here we describe the safest methodologies.

The following is pertinent for infested domestic or commercial recirculating systems housing only tropical marine teleosts. Numerous countries prohibit the use of copper-based chemotherapeutics for food fish, therefore adhere to national legislation. *Permit the remedy to diminish in flesh for at least 10 to 15 days before harvest* (Burr et al. 2012) and preclude cross-contamination/reinfection throughout. ***Painstakingly comprehend all information before treatment***.

Copper Ions: Attenuation

Nonchelated "free" copper occurs as cupric (Cu^{2+}) or cuprous (Cu^{+}) ions in aqueous solution. Only the former are therapeutic whose pK_a is 8.6 (Louis et al. 2009), therefore, the concentrations of both ions are the same at pH 8.6.

Protein skimmers export copper-organic complexes, and "free" copper also forms stable associations with bicarbonate (HCO_3^-), bromide (Br^-), carbonate (CO_3^{2-}), chloride (Cl^-), fluoride (F^-), hydroxide (OH^-), iodide (I^-), phosphate (PO_4^{3-}), and sulphate (SO_4^{2-}). Nevertheless, between pH 7.5 and 8.3, all but 20 percent of natural seawater's copper is sequestered by organics (Louis et al. 2009).

NB the abundance of copper in natural seawater is exceedingly low, so the following percentages do not represent final concentration, they merely express ionic form and relative occurrence.

Excluding manifestations with less than 4.5 percent, the percent speciation of inorganic copper at pH 8.1 in oceanwater: 66.98 percent $CuCO_3$; 18.33 percent $Cu(CO_3)_2^{2-}$; 7.78 percent Cu^{2+}, and 4.7 percent $CuOH^+$ (Caldeira & Wickett 2003, cited in Millero et al. 2009). Thanks to the absence of substrate in commercial displays (Yanong 2009; Yanong 2018), most cupric ions (Cu^{2+}) bind to the calciferous medium in pressurised sand filters (Keith 1981).

Granular activated carbon (GAC) sequesters remedies and thus cannot be used with chemotherapeutics, and as such, the concurrent maintenance of Cu^{2+} and the mandatory oxidation of phenolics relies upon the correct application of potentially hazardous ozone. Consequently, become fully familiar with its use. Consult: The Complete Reef Aquarist (Aslett 2024).

Therapeutic Dosing

Fish *must be* gradually acclimated to remedies, because ***exposing fish too rapidly***, ***embolises their secondary gill lamellae by separating epithelia from their basement membranes which invariably proves fatal*** (Fig 18.). Regardless, the biofilter consortium may expire. Formalin is a potent toxin that raises fish cortisol (Reardon & Harrell 1990; Tarkhani & Imanpoor 2012; Fredricks 2015), yet finfish and nitrifying communities will be unaffected using the approaches and concentrations recommended herein.

Rarely does the author suggest substances are administered without first ascertaining their residual concentration, where Nassiri and collaborators outlined a spectrophotometric technique measuring the abundance of formaldehyde in seawater (Nassiri et al. 2018). The entire paper is available at ResearchGate.net. However, utilising the Reef Ranch™ formulation ensures the decay of formaldehyde remains relative to that of Cu^{2+}, and thus it is merely necessary to accurately evaluate and maintain cupric ions.

100 percent formalin is a saturated solution of around 37 percent formaldehyde gas dissolved in water, which does not accumulate in the tissues of fish which makes it ideal for mariculture. Chronic human exposure leads to hypersensitivity, so wear PPE such as goggles, gloves, clothing, and a respirator, and open the container in a well-ventilated area and seal properly afterwards.

Pure formalin forms a white precipitate of paraformaldehyde at temperatures below 5°C (40°F) that kills fish on contact, whose formation is forestalled by the addition of 10 to 15 percent methanol (Francis-Floyd 1996). Formalin should be stored at room temperature in a sealed container, whereas marine fish tolerate methanol at concentrations exceeding 10 g l^{-1}, albeit preferably, fish-only systems should not comprise more than 1 g l^{-1} (Baltz et al. 2005).

A dose of copper and formalin is first diluted in a litre of fresh warm aerated R/O water, then ***administered sequentially to each holding aquarium 2 to 3 ml at a time using a disposable Pasteur pipette***, which steadily acclimates fish to the chemotherapeutic. Take your time. Medicating the displays attenuates the vulnerable stages of parasites and expedites biofilter adaptation. Herein we only endorse remedies formulated from copper sulphate ($CuSO_4$), because it is impossible to accurately determine residual malachite or coppers chelated with citric or ethylenediaminetetraacetic acids (EDTA; Yanong 2018).

Each $CuSO_4$ is bound to five molecules of water in crystalline copper sulphate pentahydrate ($CuSO_4.5H_2O$; Yanong 2018), where Cu^{2+} and SO_4^{2-} disassociate in aqueous solution with the former combining with six octahedrally-aligned water ligands (Clarke 2014). We maintain 0.05 mg l^{-1} Cu^{2+} to suppress several communicable diseases arriving on new specimens. However, we do not

attain this concentration until three days of administrations.

Starting with a system devoid of copper and formalin on the first day, the end of day target is 0.02 mg l^{-1} Cu^{2+}. *Divide into four doses* necessary to attain 0.005 mg l^{-1}, dilute each with a litre of warm aerated R/O water, and administer one to the holding aquaria every two hours as described above. All calculations *must be* performed in triplicate. *Get this wrong and animals die*; do nothing, and animals die. See: detailed calculations below.

A Cu^{2+} Merck test kit measures accurately from 0.00 to 0.05 mg l^{-1} and above in seawater (Figs 15. & 21.) which is a comparative colourimetric assay that facilitates precise cupric ion estimation below 0.05 mg l^{-1}, which is precision that becomes second nature.

Test on the morning of day two to determine overnight Cu^{2+} attenuation which is typically ~0.01 mg l^{-1} leaving a residuum of 0.01. Always test, *never assume*. An equal decay occurred every night and day for several years at the Reef Ranch™, albeit despite the significant cost of each assay, morning and afternoon tests were mandatory. Calculate the amount necessary to attain an end of day two target of 0.04 mg l^{-1}, which is likely 0.03 mg l^{-1}, and administer one quarter dose every two hours as before.

The systemic dilution will be in the region of 0.03 mg l^{-1} on the morning of day three. Test and calculate the dosage necessary to raise the concentration to 0.05 mg l^{-1} Cu^{2+} and dispense as before. Ascertain the residual concentration in the morning, and again in the afternoon from day four, thereupon medicate twice daily to maintain 0.05 mg l^{-1}. The entire dose is now diluted in a litre of water and sequentially administered in 2- to 3-ml quantities to the holding aquaria over 10 minutes. The resident fish and biofilter consortium are safely adapted, whilst the acclimation technique outlined in The Complete Reef Aquarist, ensures new arrivals are exposed safely, because suitable exporters maintain the same strength of therapeutic copper. It is vital this procedure is ultraprecise, so initially perform duplicate copper assays.

The only suitable Merck test kits cost more than 150GBP for merely 125 analyses. They are extremely accurate and reliable and only measure therapeutic Cu^{2+} in seawater. *There are no shortcuts or less costly avenues.* Besides, unless the aquatics industry is littered with proprietors with nil acumen, why keep these items in stock. This is a significant but worthwhile investment.

Leading suppliers of commercial equipment sell the appropriate test kit and remedy, therefore make enquiries or formulate the suggested copper- and formalin-based chemotherapeutic described below. Merck only delivers to commercial premises. See above: The "disease is always present" debate: myth or reality?

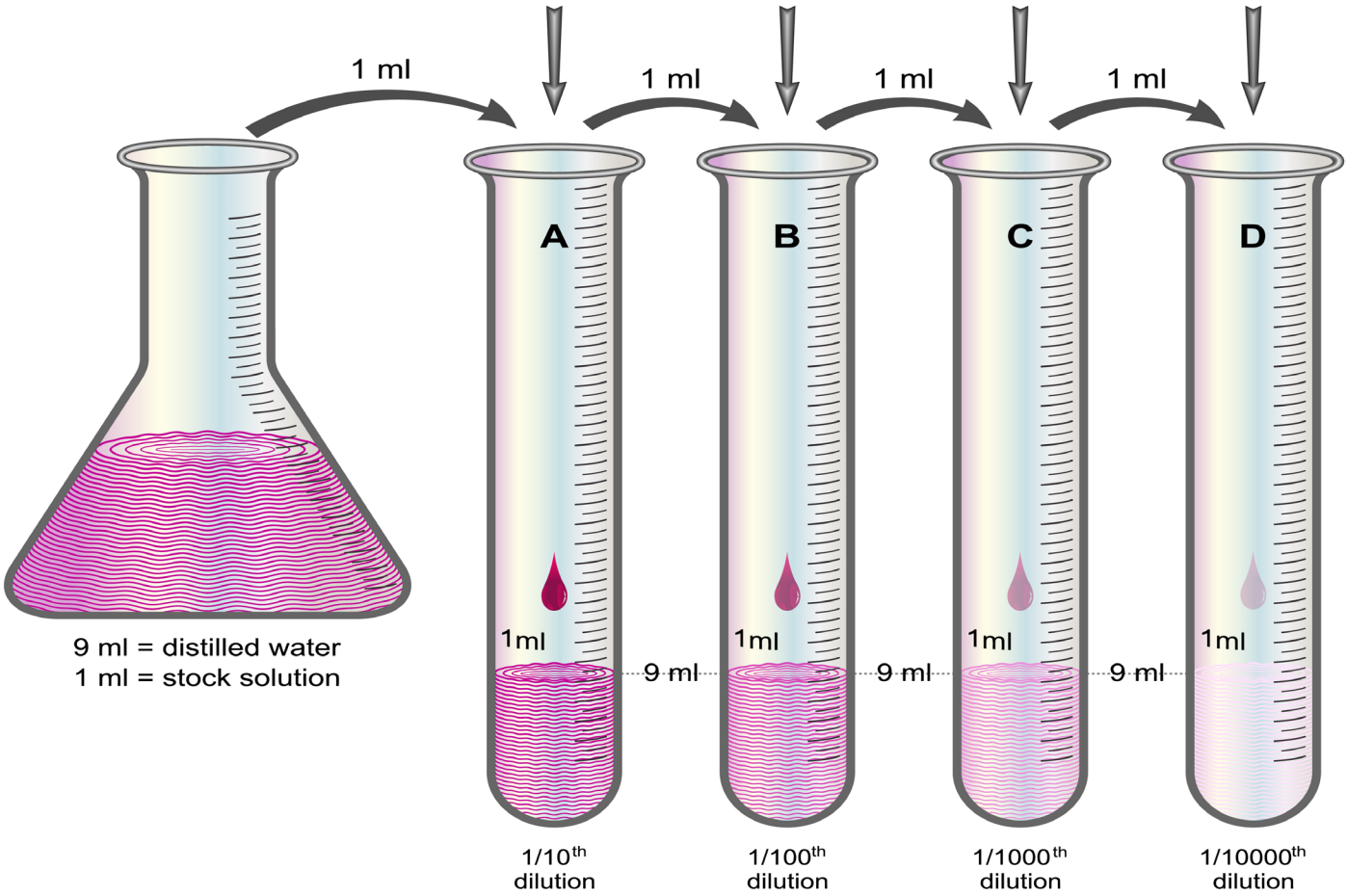

Fig 20. Serial dilutions where each step is 1 ml in 9 which generates a ten-fold dilution. Stock solutions are typically 100 times more concentrated if solubility allows, because it confers ionic stability and less costly balances may be used. All our dilutions will likely be 1 ml in 9 or 2 ml in 18. *NB* the surface of a liquid is measured from the bottom of the water's "skin" known as the meniscus.

Active Infections

Correct diagnosis is the cornerstone of any successful therapy insofar as lymphocystis virus manifests as pinkish cream cauliflower-like growths whereas eye parasites orchestrate catastrophic corneal eruptions, neither of which respond to copper. Nevertheless, Cu^{2+} mitigate *Amyloodinium ocellatum*, *Brooklynella hostilis*, *Cryptocaryon irritans*, *Uronema marinum*, and some pathogenic bacteria and harmful algal blooms (HABs). Before launching a pathology-ameliorating campaign, fully examine the system for design

imperfections and husbandry to exclude the chance that the illness is caused by stress and immunosuppression. Fish often become "spotty" after being exposed to hydrogen sulphide (H_2S). Consult: The Complete Reef Aquarist for further guidance (Aslett 2024).

If the above protocol is adopted and followed to the letter, it will likely ***irradicate all perceptible illnesses*** apart from those caused by viruses, eye flukes, gas supersaturation, or sources of H_2S. Fish systems operated without chemotherapeutics are inevitably infested, while it is challenging to eliminate an actively proliferating parasite in a recirculating system. The outlet's reputation nosedives, fish cannot be sold, and further specimens cannot be introduced, because the cupric ion concentration necessary to combat an active infection is comparatively enriched.

Mitigation

Attain and maintain 0.05 mg l^{-1} of Cu^{2+} as described above, then raise the temperature of the system in daily 1°C increments until 30 (86°F), because heat shortens parasitic life cycles which increases the rate at which the therapeutic can act on their vulnerable stages. Rapid gill irrigation at 30°C on all upright fish with nominal clinical signs suggests system flaws or poor husbandry. Ensure sufficient degas/regas, eliminate hydrogen sulphide, and safely minimise reductants. Consult: The Complete Reef Aquarist for further guidance. The findings of one study indicated 34°C disrupts the life cycle of *C. irritans* (Yoshinaga 2001). Be that as it may, the author does not endorse operating a system within that range, whilst heat-resistant strains remain unexceptional.

Slowly raise systemic Cu^{2+} over a day to 0.075 mg l^{-1} and symptoms will likely diminish. Therapeutic thresholds are confirmed by maintaining the concentration for three days during which livestock are examined for amelioration, because efficacious potencies must be maintained for an additional 14 weeks. If clinical signs persist, increase the concentration to 0.08 mg l^{-1}, and finally to 0.09, yet three-day observations are mandatory, and ***never exceed 0.1 mg l^{-1} Cu^{2+} for ornamentals***. The author has found it necessary to use 0.09 mg l^{-1} of Cu^{2+} to cure extraordinarily virulent strains of *Cryptocaryon irritans*.

After 14 weeks, permit Cu^{2+} to subside to 0.05 mg l^{-1} and steadily decrease the temperature to 25°C; however, do not maintain nominal Cu^{2+} concentrations in domestic fish-only systems but purchase stock from reputable and responsible suppliers that do.

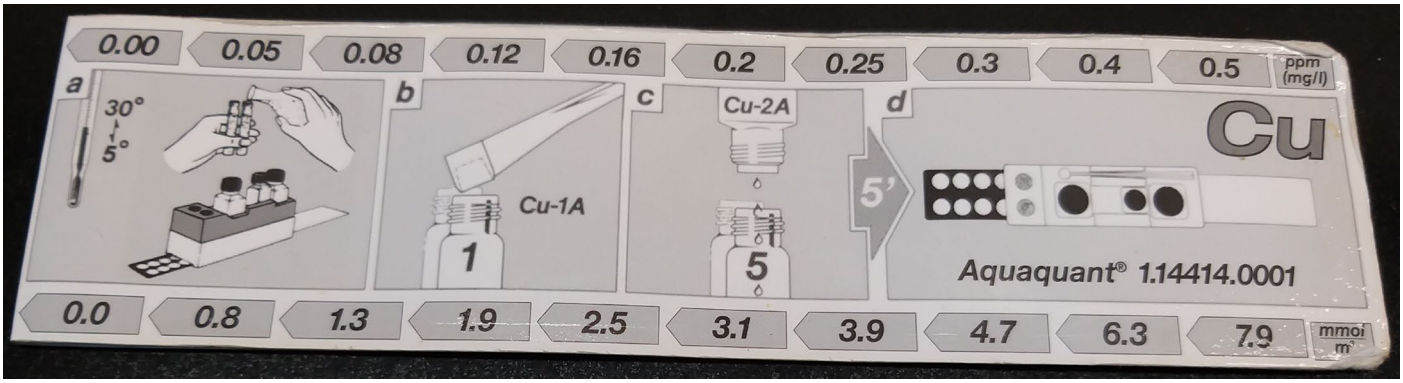

Fig 21. The resolution of the cupric ion test kit from Merck where the upper values represent mg l^{-1} (ppm).

Calculations

The following calculations and assays are only appropriate for marine remedies containing formalin and cupric ions (Cu^{2+}) administered in dedicated teleostean systems.

The correct copper and formalin based therapeutic from a leading marine supplier provides dosing guidance, which is vital information that helps us verify the concentration in the bottle, because *everything* we add to systems *must be* tested.

Dissolving copper sulphate pentahydrate in water provides merely 25.5 percent Cu^{2+}, which renders 74.5 percent of its mass superfluous (Yanong 2018).

Given that:

$$\text{Density} = \text{Mass} / \text{Volume}$$

From the bottle:

$$\text{Final Concentration (Z)} = (\text{Density of } Cu^{2+} \text{ Therapeutic} / 3.92 = X_2) / \text{System Volume (Y)}$$

$$Z = X_1 / Y$$

For example, if administering 1 ml of a solution in a system of approximately 400 litres provides 0.00005 g l^{-1} or 0.05 mg l^{-1} Cu^{2+}, then:

$$0.00005 = X_1 / 400$$

Or

$$X = 0.00005 \times 400 = 0.02 \text{ g or } 20 \text{ mg } Cu^{2+}$$

Original Formulation: $CuSO_4.5H_2O$

$$20 \times 3.92 = 78.4 \text{ mg ml}^{-1}$$

Or

$$\underline{78.4 \text{ g l}^{-1} \text{ CuSO}_4.5\text{H}_2\text{O}}$$

To confirm we must dilute the remedy sufficiently to bring the concentration within the resolution of the Merck test kit (0.05 to 0.5 mg l^{-1}; Merck product 1.14414; refill 1184590002; Figs 15. & 21.). Serial dilutions are performed in laboratories by technicians where a subsample is removed from the stock solution or our remedy and is added to a set volume of solvent and mixed, whereupon the same volume is removed from that solution and placed in an identical volume, and so on…*ad infinitum* (Fig 20.).

A 10th dilution is 1 part in 9, while pure (R/O) water weighs 1 g ml^{-1}. Therefore, water can be weighed if an accurate balance is available, or use graduated 1, 10-, or 20-ml syringes. Given that the Merck test vials hold a volume of 20 ml, either perform four 1 in 9 serial dilutions and then add a 2-ml aliquot to 18 ml in the test vial or:

Serial dilutions

1. 2 ml of 20 mg ml^{-1} Cu^{2+} is added to 18 ml = 2 mg ml^{-1}
2. 2 ml of 2 mg ml^{-1} is added to 18 ml = 0.2 mg ml^{-1} or g l^{-1}
3. 2 ml of 0.2 mg ml^{-1} is added to 18 ml = 0.02 mg ml^{-1} or g l^{-1}
4. 2 ml of 0.02 mg ml^{-1} is added to 18 ml = 0.002 mg ml^{-1} or g l^{-1} or 2 mg l^{-1}
5. 2 ml of 0.002 mg ml^{-1} is added to 18 ml = 0.0002 mg ml^{-1} or g l^{-1} (test vial)

Or

<u>0.2 mg l^{-1} Cu^{2+}</u>

…which is around the midrange of the Merck assay, and thus the test will return a value of 0.2 mg l^{-1} if the therapeutic contains 20 mg ml^{-1} (20 g l^{-1}) of Cu^{2+}.

After evaluating the concentration in the bottle, calculations must ascertain the volume of therapeutic necessary to raise systemic Cu^{2+} by 0.01 mg l^{-1}. Nevertheless, a central challenge remains precise estimation of the system volume, insofar as metres of complex pipework introduce irregularities in large centralised commercial systems.

The constant **Pi** (**π**) represents the ratio of a circle's circumference to its diameter (Zhivkovich 2013).

Cylindrical volume calculation:

$$V\ (ft^3) = \pi\ r^2(ft)\ h(ft)$$

ft = feet (inches / 12)
V = volume
π = 3.14159
r = pipework radius in feet (½ the internal diameter; ID)
h = pipe length in feet

Rectangular volume calculation:

$$\text{Volume in cubic feet } (ft^3) = \text{Length (ft)} \times \text{Height (ft)} \times \text{Width (ft)}$$

Cubic feet (ft^3) x 6.25 = UK gallons
Cubic feet (ft^3) x 7.51 = US Gallons

Convert UK or US gallons to litres by multiplying by 4.54 or 3.79.

Calculate the volume of each component using the above equations and reach a total, which is a painstaking yet worthwhile process. However, adhering to the principles in The Complete Reef Aquarist facilitates supply pump cessation where all working heads enter the sump. Ensure the system is operating at its *safe* maximum before de-energising the return pump; thereafter, holding aquaria, sump, ring-main supply, protein skimmer, and UV steriliser volumes are determined with empty drains.

Example

If the system volume is approximately 690 litres (152 UK gallons; 182 US gallons) and the remedy contains 20 mg ml^{-1} (g l^{-1}) of cupric ions, attain a concentration of 0.01 mg l^{-1}:

$$\text{Required grams of } CuSO_4.5H_2O = \text{System volume in litres} \times \text{Desired therapeutic concentration (mg } l^{-1}) \times 0.00392$$

(Yanong 2018)

$$690 \times 0.01 \times 0.00392 = 0.027 \text{ g or } 27 \text{ mg of } CuSO_4.5H_2O$$

Or

$$0.0069 \text{ g or } 6.9 \text{ mg } Cu^{2+} \textit{equivalents}$$

The above *hypothetical* remedy contains 20 mg ml^{-1} (g l^{-1}) of Cu^{2+} and we require 6.9 mg to raise the concentration in our 690-litre system by 0.01 mg l^{-1}, and every 0.1 ml of our therapeutic contains ~2 mg of Cu^{2+}.

$$6.9 / 2 = 3.45$$

$$0.1 \times 3.45 = \underline{0.345 \text{ ml}}$$

To raise the concentration of our 690-litre system by 0.01 mg l^{-1} we require 0.345 ml of our *hypothetical* therapeutic, therefore, attaining a concentration of 0.05 mg l^{-1} will require 0.345 x 5 = 1.73 ml. *NB* the concentration cannot be raised safely by this much in one day. See above: Chemotherapeutics: critical information proceed with caution.

The marine cupric ion assay from Merck is the only test kit recommended for maintaining cupric ion (Cu^{2+}) concentrations in seawater.

Reef Ranch™ Copper/Formalin Remedy: Commercial and Domestic Formulations

Aqueous **Cu^{2+}** become complexed with six water ligands with octahedral symmetry $\{Cu(H_2O)_6\}^{2+}$ (Clarke 2014); however, only 25.5 percent of copper sulphate pentahydrate ($CuSO_4.5H_2O$) becomes Cu^{2+} in water, and thus any calculations are corrected using the conversion factor 3.92 (Yanong 2018). Moreover, continuous immersion in 0.09 to 0.18 mg l^{-1} of formalin is safe according to Reardon & Harrell 1990, Francis-Floyd 1996, Fajer-Ávila et al. 2003, Yanong 2018, and Aslett 2024.

Using the desired administration approach of 1 ml per 80 litres of domestic system water to attain 0.05 mg l^{-1} Cu^{2+}:

Required $CuSO_4.5H_2O$ g = Volume (l) x Desired concentration of Cu^{2+} g l^{-1} x 3.92

(Yanong 2018).

80 x 0.00005 x 3.92 = 0.0157 g or 15.7 mg $CuSO_4.5H_2O$

1 ml must provide a working concentration of 0.05 mg l^{-1} Cu^{2+} for every 80 litres, therefore we need a bottle concentration of 15.7 mg ml^{-1} or

15.7 g l^{-1} $CuSO_4.5H_2O$

Calculate the number of millilitres of **formalin** required per litre. Suitable formalin contains 10 percent methanol which forestalls its precipitation to paraformaldehyde (Francis-Floyd 1996), and hence its final volume must be 10 percent greater. Formalin also becomes turbid due to the precipitation of trioxymethylene and its spontaneous decomposition to formic acid. It is therefore necessary to raise its pH a fraction above 4 using a solution of sodium hydroxide (NaOH; Sigma 2016). Formalin is oxidised to formic acid whilst cupric ions become nontherapeutic cuprous (Cu^{+}) in alkaline conditions above 100°C (212°F; Byerley & Teo 1968) and thus do not exceed pH 5. Consult: The Complete Reef Aquarist for guidance.

A US and UK gallon are 3.78541 and 4.54609 litres

Francis-Floyd and Pouder specified that 1 ml of formalin in every 10 US gallons is the equivalent of 25 mg l^{-1} after correcting for methanol (Francis-Floyd & Pouder 2019), whereas another study suggested 0.95 ml (Masser & Jensen 1991). Spontaneous oxidation of formaldehyde to formic acid diminishes dissolved oxygen (DO) by 1 mg l^{-1} for every 5 mg l^{-1} of formalin added to freshwater (Masser & Jensen 1991) which is likely worsened at seawater alkalinities (Byerley & Teo 1968).

25 mg l^{-1} of formalin originates from every 1 ml added to 10 US gallons (Masser & Jensen 1991; Francis-Floyd & Pouder 2019).

Convert to UK gallons:

4.54609 / 3.78541 = 0.2; UK gallons are 0.2 times greater.

Therefore 1.2 ml of formalin for every 10 UK gallons (45.46 litres) yields 25 mg l^{-1}.

We require 1 ml of our remedy to generate our target concentration for every 80 litres of system volume.

For every 10 UK gallons:

80 / 45.4609 = 1.76

1.2 x 1.76 = 2.11 ml of formalin will raise the concentration of 80 litres of system volume to *a* ***likely lethal*** 25 mg l^{-1}.

Our target suppressing concentration of formalin is 0.09 mg l^{-1}.

25 / 0.09 = 278

2.11 / 278 = 0.0076 ml of formalin will provide a working concentration of 0.09 mg l^{-1} within 80 litres of system volume.

= 7.6 ml l^{-1} of formalin

Comprehensive Formulation (l^{-1})

The solubility of $CuSO_4.5H_2O$ is greater than or equal to 148 g l^{-1} at 0°C (32°F; Hartley & Kidd 1987); however, store at room temperature and shake well before use.

It is suggested that the above concentrations be increased 10-fold ***for commercial*** purposes, therefore 157 g $CuSO_4.5H_2O$ is dissolved in 924 ml of R/O water and 76 ml of formalin.

1 ml for every 800 litres will provide:

0.05 mg Cu^{2+} and 0.09 mg of formalin l^{-1}

A 500 UK gallon system will eventually require:

500 x 4.54 = 2,270 litres

2,270 / 800 = 2.84 ml

15.7 g of $CuSO_4.5H_2O$ is dissolved in 992.4 ml of R/O water and 7.6 ml of formalin ***for domestic*** *use*.

1 ml for every 80 litres will provide:

0.05 mg Cu^{2+} and 0.09 mg of formalin l^{-1}

A 60 UK gallon system will eventually require:

60 x 4.54 = 272.4 litres

272.4 / 80 = 3.4 ml

The above chemotherapeutics have been subjected to rigorous toxicological analyses and safely administered in both domestic and commercial *fish-only* settings whose shelf-life should exceed two years. ***NB the target concentration of Cu^{2+} for day one should be 0.02 mg l^{-1}.*** See above: Chemotherapeutics: critical information proceed with caution.

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