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Natural versus synthetic anesthetic for transport of live fish: A review

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ABSTRACT

An increased awareness of food safety and quality has led to an increased demand for live edible fish. This product requires effective and efficient handling technology and transport to ensure a low mortality rate, but live fish transport has not yet met this criteria. Anesthetized fish can be transported in larger quantities without being stressed during transfer, maintaining a higher quality. This paper explored synthetic and natural anesthetics derived from terrestrial and aquatic resources. This review of the different types, sources, and applications of natural and synthetic anesthetics was used to investigate the potential of seaweed as an anesthetic. In addition, advantages and disadvantages of anesthetics and the potential of seaweed as a prospect of anesthetic are discussed.

1. Introduction

Current demand for live edible fish and ornamental fish is increasing. Ornamental fish, sold for their beauty, need to be alive for sale, so maintaining their viability during transportation is crucial. Species of popular ornamental fish include: bettas (*Betta splendens*), goldfish (*Carassius auratus*), guppy (*Poecilia reticulata*), zebra fish (*Brachydanio rerio*), and common carp (*Cyprinus carpio*) (ITPC, 2014). Meanwhile, high demand for edible fish has resulted from a rise in consumer awareness of healthy food (Prastiti, 2017). These increases have occurred in several commercially-important species, such as giant tiger prawn, whiteleg shrimp, grouper, common carp, tilapia, and catfish. Live commodities are sold at a higher price than fresh and frozen commodities. Thus, a study about handling and transportation of live fish for a higher viability of commercial fish is essential.

The transport of live fish in Indonesia has not been efficient in terms of technology and cost. The development of transportation technology to produce a higher viability of commercial fish at a rational price is required. One potential method involves anesthetizing fish before transportation. Transportation is a strong trigger for stress in fish (Manuel et al., 2014), with atypical environmental conditions, including temperature, pressure, and agitation. Stress leads to decreased immune system function, resulting in sickness and death (Tacchi et al., 2015). Conversely, anesthesia reduces metabolic rate, need for oxygen, activity, and response to stress (Brown, 2011; Skar, Haugland, Powell1, Heidrun, & Samuelsen, 2017), enabling fish to be transferred in higher densities more efficiently. Anesthesia has been used to handle and transport live fish for years in biology and aquaculture studies in order to reduce stress and relax fish (Akbari, Khoshnod, Rajaian, & Afsharnasa, 2010; Manuel et al., 2014; Septiarusli, Haetami, Mulyani, & Dono, 2012). There are two types commercial anesthetics: natural and synthetic agents. Synthetic agents are banned because of safety issues and residues, while natural agents are more developed and expected to have a bright prospect in the future. Several synthetic agents frequently used to anesthetize fish include: tricaine methanesulfonate (MS-222) (Bahrekazemi & Yousefi, 2017; Ghanawi, Samer, & Imad, 2013; Skar et al., 2017), phenoxy ethanol (Bahrekazemi & Yousefi, 2017; Ghanawi et al., 2013), and etomidate (Readman, Owen, Toby, & KnowlesMurrell, 2017; Ross & Ross, 2008).

Anesthetics commonly derived from terrestrial plants include clove oil (*Eugenia aromatica*) (Abdolazizi, Ghaderi, Naghdi, & Kamangar, 2011; Aydın, Akbulut, Kucuk, & Kumlu, 2015; Okey, Keremah, & Gabriel, 2018; Sindhu & Ramachandran, 2013; Sutili, Gressler, & Baldisserotto, 2014) seeds from the fish poison tree (*Baringtonia asiatica*) (Septiarusli et al., 2012), rubber seeds (*Hevea brasiliensis*) (Hasan, Farida, & Ertiyasa, 2016), and *tuba* roots (*Derris elliptica*) (Irawan, Efendi, & Ali, 2014; Prariska, Tanbiyaskur, & Azha, 2017). Among a number of natural agents, clove oil is the most common and has a reputation as the most effective fish anesthetic (Kamble, Saini, & Ojha, 2014; Sutili et al., 2014), with eugenol as its active compound.

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However, there is no study on the effect of its exposure to fish flesh when consumed. Clove oil is safe, but is not recommended by the FDA as fish anesthetic. A disadvantage of clove oil as an anesthetic is that its therapeutic index is relatively low for fish safety (a narrow safety limit), so a slight difference in dose used may result in fish death or a slow recovery time for surviving fish (Kamble et al., 2014). Thus, studying safe agents for fish anesthesia that allow for fast recovery (Kamble et al., 2014), effectiveness at a low concentration, a low residue in tissue, and a low cost is essential in order to develop “green anesthetics” with lower risk to the environment and health (Ramanayaka & Atapattu, 2006; Rezende, Pascoal, Vianna, & Lanna, 2017).

Aquatic resources can be utilized for the exploration of safe anesthetics. Seaweed is an aquatic commodity with anesthetic potential. Seaweed has not been widely studied nor acknowledged as a potential natural anesthetic, unlike terrestrial plants, which have demonstrated hypnotic or anesthetic effects. Reviews regarding the bioactivity of seaweed are limited to antibacterial (Hanapi, Fasya, Mardiyah, & Miftahurrahmah, 2013), antioxidant (Kania et al., 2013), and anticancer (Namvar et al., 2012) capabilities, with none for the anesthetic properties of seaweed, particularly for the purpose of fish transportation. This review explores synthetic and natural anesthetic types, applications, and their respective advantages and disadvantages. In addition, this paper discusses seaweed as a potential natural anesthetic for live fish transportation.

2. Live fish transportation

Live fish transportation involves impelling fish to be placed in a different environment from its origin, a process accompanied by sudden changes in environmental conditions. Live fish transportat increases the density of fish while keeping the fish in good condition, so as to achieve a high survival rate upon arrival at destination. Poor water exchange is a crucial factor that results in decreased water quality metrics, such as reduced oxygen through the accumulation of suspended solids, ammonia, and carbon dioxide (Emmanuel, Fayinka, & Aladetohun, 2013). In addition to water quality, the success of transportation depends on other factors including time, temperature, density, size (Belema et a., 2017), oxygen, and duration of transportation (Imanto, 2008). The inefficient transportation process creates a high mortality rate, encouraging authors to research and explore more efficient and effective fish transportation technology.

3. Fish anesthesia

When fish are anesthetized, they are in an unconscious condition generated by a controlled process that decreases sensitivity to external stimuli. Anesthesia can reduce general activity, stress or pain, metabolic rate and oxygen demand, and improve ease of handling (Ross & Ross, 2008; Martins, Diniz, Feliz, & Antunes, 2018). The anesthetic process uses various types of both natural and synthetic agents. The ideal anesthetic should produce rapid anesthesia (1–5 min) and rapid recovery (< 5 min), and be cheap, practical to use, water soluble, and leave no residue in fish, humans, or the environment (Brown, 2011; Marking & Meyer, 1985; Ross & Ross, 2008; Weber, Peleteiro, García, & Aldegunde, 2009).

3.1. Chemical anesthetic agents

The most commonly used synthetic anesthetics include tricaine methanesulfonate (MS-222) (Weber et al., 2009), benzocaine (etil paraaminobenzoate) (Kiessling, 2009), 2-phenoxyethanol, eetomidate (Ross & Ross, 2008; Weber et al., 2009), and carbon dioxide. Some applications of anesthetics include Sumatran Fish (*P. filamentosus*) (Pramod, Ramachandran, Sajeevan, Thampy, & Pai, 2010; Zahl, Samuelsen, & Kiessling, 2012, and *Baronang* (Ghanawi et al., 2013).

3.1.1. Tricaine methanesulfonate (MS-222)

The MS-222 compound (Tricaine methanesulfonate = $C_9H_{11}O_2N + CH_3SO_3H$) is a compound with a basic molecule of ethyl 3 aminobenzoate, present in a white crystalline powder. Several studies have reported the effectiveness of this compound in a function as fish anesthesia Zahlet al., 2012; Ghanawi et al., 2013). The MS-222 concentration of 10–15 mg/L was used for handling *S. rivulatus* (Ghanawi et al., 2013). MS-222 is also reported to be the most effective and ideal anesthetic agent for *Hippocampus* in coping with stress, survival, and production efficiency (Pawar et al., 2011). The anesthetic function of MS-222 is effective for Atlantic halibut (*Hippoglossus hippoglossus*) (Zahl et al., 2012). Concentrations up to 40 mg/L are considered safe during long-term exposure to young cobia fish (*Rachycentron canadum*) (Gullian & Villanueva, 2009). The MS-222 compound is also used as an antidepressant during the transportation of Sumatran fish (*P. filamentosus*), improving their post-transport viability and reducing economic losses (Pramod et al., 2010). The MS-222 has proven to be effective for carp, rainbow trout (Readman et al., 2017), and adult zebrafish (Martins et al., 2018). However, it is more dangerous, with reports that chronic exposure to fish, amphibians, and humans can cause eye and neurological disorders, but recent incidents have not been reported (Brown, 2011).

3.1.2. Benzocaine compounds

This compound is identical to MS-222 and is white, odorless, and tasteless. The dose is almost same as MS-222. This anesthetic is less expensive than other available anesthetic compounds (Gomes, Chippari-Gomes, Lopes, Roubach, & Araujo-Lima, 2011) and has been used for the handling and transport of *S. rivulatus* species at doses ranging from 5 to 10 mg/L (Ghanawi et al., 2013). Benzocaine is also effective as an anesthetic for the Sumatran fish (*P. filamentosus*) (Pramod et al., 2010) and Atlantic halibut (*Hippoglossus hippoglossus*) (Zahl et al., 2012), and reduces economic losses during transport. Anesthetizing *Carassius carassius* requires an optimum dose of 100 ppm (Heo & Shin, 2010).

3.1.3. Phenoxyethanol

Phenoxyethanol is a clear, colorless, slightly odorless liquid and readily water soluble. It is applied as a topical anesthetic, bactericide, and fungicide, which is useful for laparotomy or abdominal surgery, at a relatively low cost (AQUI-S, 2013; Brown, 2011; Ross & Ross, 2008). This anesthetic agent is most commonly used in aquaculture (Serezli, Basaran, Gungor, & Kaymakci, 2012) because it is economical and effective in its preparation and effects (Nair & Williams, 2015; Serezli et al., 2012; Weber et al., 2009). The phenoxyethanol compound is more suitable for *S. rivulatu* (Ghanawi et al., 2013). The results showed an effective dose of phenoxyethanol ranged from 0.2 to 0.6 mL/L (Tsantilas et al., 2006; Perdikaris et al., 2010).

3.1.4. Etomidate

Etomidate is an anesthetic water soluble powder that has been widely used by fish researchers. Anesthesia time was achieved within 1–2 min without hyperactivity, resulting in a faster recovery compared to using an MS-222 anesthetic (AQUI-S, 2013; Ross & Ross, 2008). Some researchers use this anesthetic to soothe fish during transportation, such as Atlantic salmon (*Salmo salar*) (Finstad, Iversen, & Sandodden, 2003).

3.2. Natural anesthetic agents

3.2.1. Terrestrial resources

Researchers have reported some terrestrial plants contain anesthetic substances. *Barringtonia asiatica* seed extract (14 mg/L) was reported to contain saponin compounds that could stun a grouper fish so that it can be transported without a water medium for 6 h with a survival rate of 80% (Septiarusli et al., 2012). Research on tuba root toxicity (*Derris*

Table 1
Applications of natural and synthetic anesthetics.

Fish name	Size		Anesthetic	Dose (mg/L)	Induction Time (minute)	Recovery Time (minute)	Literature
	Weight (g)	Length (cm)					
Notopterus chitala	400–600	–	Nutmeg extract (*Myristica fragnans* Houtt)	7	7	–	Dayatino, Raharjo, and Rachimi (2014)
Goldfish (*Carasius auratus*)	0.23 ± 0.03	2.41 ± 0.56	MS-222	200	2.24 ± 0.95	3.09	Kucuk and Coban (2016)
Oreochromis niloticus	40.8 ± 1.20	13.60 ± 0.20	*Alpinia galanga* oil	700	4.28	7.3	Pikulkaew, Khumpirapang, Chaisri, and Okonogi (2017)
Oreochromis sp.	56.3 ± 14.30	–	Eugenol	100	1.16	3.35	Rucinque, Polo, Borbón, and Mantilla (2017)
			Benzocaine	100	2.88	2.81	
Hyphessobrycon sp1	–	28.20 ± 3.06	Clove oil	100	0.19 ± 0.08	6.03 ± 3.1	Fernandes et al. (2017)
Hyphessobrycon sp.2	–	31.70 ± 2.00			0.28 ± 0.29	5.3 ± 2.7	
Hemigrammus sp	–	29.60 ± 5.13			0.23 ± 0.05	6.2 ± 2.9	
Oreochromis niloticus	18.45 ± 3.20	–	*Ocimum gratissimum* oil	60	3.51 ± 0.34	3.35 ± 0.27	Adewale, Adeshina, and Yusuf (2017)
Guppy (*Poecilia vivipara*)	1.42 ± 0.94	3.97 ± 0.10	MS-222	100	1.63 ± 0.52	1.81 ± 0.26	Bolasina, Azevedo, and Petry (2017)
				200	0.55 ± 0.18	2.00 ± 0.41	
			Benzocaine	100	1.34 ± 0.35	2.05 ± 0.51	
				200	0.60 ± 0.14	2.27 ± 0.58	
			Eugenol	100	1.87 ± 0.64	1.78 ± 0.86	
				200	1.47 ± 0.79	2.73 ± 0.95	
Oryzias dancena	0.33 ± 0.06	3.08 ± 0.35	Lidocaine-HCl	800	1.25 ± 0.20	2.2 ± 0.34	Park et al. (2017)
			Clove oil	125	1.35 ± 0.19	2.39 ± 0.35	
Oreochromis niloticus	24.1 ± 3.80	9.1 ± 1.78	Tea tree oil	1200	4.58 ± 1.12	6.12 ± 1.57	Rezende et al. (2017)
			Clove oil	1239	1.95 ± 1.07	21.13 ± 0.77	
			Eucalyptus oil	1217	20.52 ± 4.30	21.00 ± 5.85	
			Mint oil	1199	8.80 ± 0.30	8.93 ± 2.93	
Epinephelus akaara	14.3 ± 4.21	35.1 ± 5.92	Clove oil	75	0.93 ± 0.13	3.82 ± 0.20	Park, Lee, and Lim (2018)
	1044 ± 149.63			75	1.18 ± 0.16	1.38 ± 0.15	
Clarias gariepinus	3.26 ± 2.30	8.34 ± 1.82	Clove powder	80–140	3.00	5.00	Okey et al. (2018)
Heterobranchus bidorsalis	2.89 ± 1.80	7.55 ± 1.04					
Lesser guitarfish *Zapteryx brevirostris*	541.16 ± 119.18	44.47 ± 4.48	Eugenol	85	3.00	4.00	Takatsuka et al. (2018)

elliptica) with rotetone compounds for stunning fish has also been reported (Irawan et al., 2014; Olufayo, 2009). Laminarin in rubber seed extract (*Hevea braziliensis*) can anesthetize *Chanos chanos* Forskal at a dose of 5 mL/L and produce a 100% survival rate (Hasan et al., 2016). The nutmeg extract (*Myristica fragans*) has been utilized for ornamental fish, such as Platy (Johny & Inasu, 2016) and goldfish (Al-Niaeem, Mohammed, & Al-hamadany, 2017). Cashew leaf extract (*Psidium guajava*) at a concentration of 0.25% is effective as an anti-stress agent for tilapia transport (Suwandi, Nugraha, & Zulfamy, 2013). Nevertheless, some researchers insist that the most effective natural agent used for fish-anesthesia is clove oil (*Eugenia aromaticum*) (Aydın et al., 2015; Fernandes, Bastosb, Barretob, Lourençoc, & Penhab, 2017).

Eugenol is the main ingredient of clove oil (range 70%–90%), distilled from flowers, stems, and leaves of *Syzygium aromaticum* cloves (*Eugenia aromaticum* or *Eugenia caryophyllata*) (Putri, Hidayat, & Rahmah, 2014), has several uses in dentistry and medicine, serving as an antiseptic, analgesic, and anesthetic drug (National Toxicology Program, 2002). A high efficacy at a low dose, non-specific toxicity, and cost efficiency are benefits of clove oil use in aquaculture and aquatic studies (Fernandes et al., 2017; Kamble et al., 2014). Clove oil proved to be an effective anesthetic agent for fisheries management research on invasive and non-invasive species (Sindhu & Ramachandran, 2013).

Cyprinus carpio (Kamble et al., 2014; Saini, Kamble, Ojha, & Raosaheb, 2018), *Solea senegalensis* (Weber et al., 2009), *Oncorhynchus mykiss* and *Carassius auratus* (Perdikaris et al., 2010), *Barbus grypus* (F Oğretmen et al., 2016), *Hyphessobrycon* and *Hemigrammus* (Fernandes et al., 2017) are examples of clove oil applications. Eugenol (clove oil) is a safe anesthetic for treating *Oreochromis niloticus*, Linnaeus (Ribeiro, Filho, Melo, & Luz, 2015) and lesser guitarfish *Zapteryx brevirostris* (Takatsuka, Costaa, Oliveiraa, Sanchesa, & Azevedoa, 2018). Based on its anesthetic formulation, eugenol is a safe and effective anesthetic that reduces stress level of *M. rosenbergii* (Saydmohammed & Pal, 2009); *Oreochromis niloticus* (Simões, Lombardi, Gomide, & Gomes, 2011; Ogretmen et al., 2014) and grass shrimp (*Palaemonetes sinensis*) (Li et al., 2017). In general, clove oil is cheap, safe, and easily regulated (Cho et al., 2012). However, clove oil has several disadvantages, such as a low therapeutic index (Abdolazizi et al., 2011), relatively long recovery time, adverse effects on fish flavor (Sutili et al., 2014), and possible mortality in small shrimps (Li et al., 2017). Therefore, it is necessary to study the natural agents which have a more effective recovery time with less residue to maintain taste of the fish. Seaweed is a potential natural agent, with a wide extent of bioactivities and wide applications.

3.2.2. Aquatic resources: seaweed

The potential of aquatic resources for a deeper exploration of the bioactivity possessed by commodities, such as seaweed. Information on the use of anesthetic agents from aquatic resources is still very limited for both activities and applications. Although there are many exploratory uses of seaweed and their bioactivity in traditional medicines, the associated anesthetic properties have not been widely explored (Cho et al., 2012). Sukarsa (2005) reported species of *Caulerpa* sp. could be used to stun grouper. Species of seaweeds proven to have anti-depressant activity are brown seaweed *Inyegaria stellate* (Bushra, Rahila, Iqbal, & Somia, 2012) and *Eclonia cava* (Cho et al., 2012). However, there are no reports related to anesthetic applications.

Some seaweeds with potential as an anesthetic, but still have limited information, such as those relating to the sedative effect (anesthesia) in from *Eclonia cava* (Cho et al., 2012) and antidepressant activity from brown algae *Inyegaria stellate* (Bushra et al., 2012). *E. cottonii* seaweed contains a variety of active compounds, such as alkaloids, flavonoids, phenolics, steroids, tannins, and saponins (Sharo, Ningsih, Nasichuddin, & Hanapi, 2013; Afif, Fasya, & Ningsih, 2015; Rahayu., 2015), which are sedative or hypnotic and toxic to fish (Harborne,

Table 2
Comparison between natural and synthetic ingredients.

Parameter	Anesthetic		Description
	Natural	Synthetic	
Activity	Higher		
Residue	No residues found	Residues in fish and humans	
Environmental impact	Relatively fewer environmental impacts	Negative impacts of the residues	
Price/cost	More affordable	Relatively more expensive (Cho et al., 2012)	Some types are derived from waste plants
Dose effectiveness	More effective for lower concentrations		Although in general it tends to be the same
Impact on taste/fish flesh	Some types, such as clove oil having an impact on flesh taste of the stunned fish	Some reported tasteless	Not many reports discussing impacts of chemical ingredients on the flesh taste

1987; Rahayu, 2015; Rohyani, Aryanti, & Suripto, 2015). The existence of these compounds qualifies *E. cottonii* as a prospect for an anesthetic. This is supported by results from a best toxicity test in *E. cottonii,* linked to its bioactivity, which reported a value of 58.01 mg/L with ethanol solvent (Sharo et al., 2013) and 70.32 mg/L with n-butanol (Afif et al., 2015). The toxicity levels show that *E. cottoni* is toxic despite having a wide range of bioactivities. Meyer et al. (1982) similarly reported that if an extract has an LC_{50} value < 1000 mg/L, it has toxic activities, whereas if an extract has an LC_{50} value of 30–200 mg/L, it has antimicrobial activities (Winarno, 2002).

In addition, larvicidal activity of *Caulerpa racemosa* seaweed (Nagaraj & Osborne, 2014) and terpenes from *Laurencia dendroida* (Neto et al., 2016) is important, because the active compounds that have larvalidal activity in terrestrial plants have anesthetic or sedative activity. Thus, seaweed, which has a larvacidal activity, potentially has an anesthetic or antidepressant activity.

4. Application and comparison of natural and chemical anesthetics

Applications of natural and synthetic anesthetics have been reported by previous researchers, summarized in Table 1. Conforming to coverage on application of anesthetics derived from natural and chemical resources, the comparison between those, along with respective advantages and disadvantages are summed in Table 2.

5. Discussions and concluding remarks: prospects of anesthetics for transportation of live fish in the future

Tables 1 and 2 indicate that natural anesthetics are relatively equivalent to synthetic anesthetics in terms of the time of induction and recovery, which, for good anesthesia, is less than 5 min. Clove oil and eugenol were relatively more efficient natural anesthetic agents compared to synthetics, having faster induction and recovery times. This is supported by Sutili et al. (2014)'s assertion that clove oil is still the most effective fish anesthetic. Although synthetic anesthetics are better than natural anesthetics in some cases, residues and expensive prices are important considerations.

These considerations, and other respective advantages and disadvantages of natural and synthetic anesthetics, predict that natural anesthetics are more likely to be explored and used in the future. This is also supported by a raised awareness of healthy lifestyles and quality assurance of products for consumption, in addition to avoiding exposure to residues from synthetic anesthetics. Consequently, studies on different natural anesthetics to replace synthetic anesthetics for transportation of live fish are expected to be carried out more broadly, with novel anesthetics more effective and efficient in terms of technology and cost.

Natural and synthetic anesthetics produce the same time of induction and recovery. Doses for the same type of fish are relatively lower for natural agents. Natural anesthetics can be used as a substitute for synthetic anesthetics and are safer for fish objects and consumers. Future studies will identify more effective, safe, and inexpensive natural anesthetics for economically important fish species of various sizes and ages, both ornamental and for consumption.

The challenge is to discover environmentally friendly natural anesthetics with strong effect, low costs, and no negative effects on stunned fish. Sources of anesthesia were terrestrial and aquatic plants and exploration of aquatic products, both freshwater and saltwater, for anesthetics is predicted to develop in the upcoming years, as some studies have demonstrated that the bioactivities of aquatic products are better than terrestrial products. Seaweed, abundant in species, is one source that has potential to be explored. Research on other species to investigate similar bioactivity must be conducted. Increased public awareness regarding quality and food safety is another supporting factor for the need for natural anesthetics that can be used in the transport of live fish.

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