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Experimental evaluation of co-culture of juvenile sea cucumbers, Holothuria scabra (Jaeger), with juvenile blue shrimp, Litopenaeus stylirostris (Stimpson)

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Keywords: polyculture, holothurian, sandfish, ammonia, burrowing behaviour

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Abstract

The co-culture of juvenile sea cucumber *Holothuria scabra* (Jaeger), or 'sandfish', with juvenile blue shrimp *Litopenaeus. stylirostris* (Stimpson) was tested by growing groups in co-culture and monoculture for 3 weeks in tanks with enriched sand substratum. Feed was supplied on trays, accessible only to shrimp. Survival of shrimp and sandfish was high in all treatments (73^ 100%). Growth of shrimp did not differ between monoculture and co-culture, but sandfish grew significantly slower in co-culture (P 50.03), although their sand burrowing and surface foraging were apparently unaffected by shrimp (P 50.76). However, shrimp increased the levels of total ammonia-N in tanks, which related inversely with sandfish growth (P 50.04). Conversely, sandfish did not appear to lower the water quality for shrimp culture. While sandfish bioturbate sediments and eat organic deposits, the juveniles did not significantly reduce the organic content of sand in tanks. Co-culturing juveniles of the two species in earthen ponds appears feasible, with no detriment to shrimp production, presenting a cost-effective method for growing sandfish to larger sizes for restocking. These findings underpin further studies to test the viability of commercial co-culture of sandfish with blue shrimp at later stages in the production cycle of shrimp.

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Introduction

Sea cucumbers are viable candidates for land-based aquaculture, restocking and stock enhancement. A number of species are highly valued in dried food markets in East Asia. Prices for the dried product, called 'bêche-de-mer'or 'trepang', have increased dramatically since 1990, fuelling the over fishing of wild stocks in many countries. Culture methods have been developed only recently for the purpose of restocking or commercial production (Battaglene 1999; James 1999; Liu, Zhu, Zhao, Wang & Gu 2004; Mercier, Hidalgo & Hamel 2004). At least two species of sea cucumbers are now produced commercially, using earthen ponds for grow-out; *Apostichopus japonicus* in China (Chen 2003; Chang, Yu & Song 2004) and *Isostichopus fuscus* in Ecuador (Mercier et al. 2004). Intensive monoculture of these species has arisen as shrimp farmers have looked to culture alternative species after recent viral outbreaks in shrimp culture (Chen 2003).

The'sandfish' *Holothuria scabra*, the most valued of tropical sea cucumber species, inhabits sandy and muddy coastal habitats (Hamel, Conand, Pawson & Mercier 2001). This species has been produced in relatively small numbers for experimental work, but no technical constraints impede large-scale production (Battaglene & Bell 2006). Sandfish are cultured in tanks until they reach 1g in weight, when they are grown on sand or muddy substrates (Battaglene 1999; Battaglene, Seymour & Ramofafa 1999; Pitt 2001), but their growth soon becomes density limited (Battaglene et al. 1999). Grow-out of sandfish in earthen ponds could effectively provide the large area needed to grow them to larger juvenile sizes for restocking into the wild (Pitt 2001; Pitt & Duy 2004; Purcell 2004), but the cost of earthen ponds is a constraint. Alternatively, growing sandfish juveniles in existing shrimp ponds could be cost effective because pond costs and management are already met. The use of shrimp waste would save costs of producing sea cucumbers of larger sizes, an approach shown to improve the cost effectiveness of growing American oysters (Jakob, Pruder & Wang 1993).

The potential co-culture of sandfish with shrimp provides opportunity for bioremediation of pond wastes. In coastal habitats, sea cucumbers play a role in recycling of nutrients and bioturbation of sediments (Uthicke 1999, 2001). They feed selectively on nutrient-rich detritus in sediments (Hammond 1983), so their feeding habits are unlikely to be detrimental to shrimp culture. Waste removal and sediment bioturbation by sandfish could potentially reduce nutrient loads and stratification of sediments.

In the tropical south Pacific, the blue shrimp *Litopenaeus stylirostris* (Penaeus) is a key aquaculture species. Correspondingly, the technology to produce sandfish at high latitudes presents opportunities for co-culture in ponds. *Litopenaeus stylirostris* is grown from the post-larval stage in earthen ponds in New Caledonia, where, in 2002, 12 farms produced 1800 metric tonnes of which 1300 tonnes were exported. The aquaculture industry has not yet diversifed commercially and relies on the high returns of *L. stylirostris* to cover the high costs of managing earthen ponds.

In New Caledonia, a WorldFish Center project is developing methods for releasing sandfish for restocking wild populations. Co-culture of sandfish with blue shrimp could provide an inexpensive way to grow large numbers of sandfish juveniles for release or as a commercial augment to the shrimp industry. Testing the viability of co-culture of shrimp with sandfish must be a staged process because sandfish burrow into sediments, thus experimental units must be destructively sampled to determine survival at each developmental stage of shrimp. In the present study, we tested the viability of co-culture at the early juvenile stage of both sandfish and blue shrimp. Three weeks was sufficient time to compare biotic and biochemical effects in co-culture with monoculture. We aimed to reflect the conditions in earthen ponds by using a sediment substrate (albeit coarse) in tanks and using the same unfiltered water as for ponds and limited water exchange. Feed pellets were provided to shrimp on feed trays, a practice used after stocking post-larvae *L. stylirostris* into ponds, to separate the effect on sandfish from interactions with shrimp or access to shrimp faeces from effect because of uneaten pellets.

Material and methods

Animal culture and tank preparation

Juvenile sandfish (*H. scabra*) were cultured at the IFREMER Saint-Vincent Aquaculture Research Station, New Caledonia, France. Spawning induction of 17 male and 14 female broodstock was carried out using thermal stimulation in December 2002. Larval culture and juvenile rearing followed the methods described by Battaglene (1999), James (1999) and Pitt (2001). Juvenile sandfish were grown on sand substrate in raceway tanks before the experiment. A visual examination under a dissecting microscope, following a protocol for checking for fungal or bacterial infection, discolouration and malformation, showed that the batch of juveniles was healthy and free of noticeable disease.

Juvenile blue shrimp, *L. stylirostris*, were produced at the same site from captive broodstock and reared following the methods described by Ottogalli, Galinie and Goxe (1988) and Ottogalli (1992). Young post-larval shrimp (PL1) were grown on microparticulated feed for shrimp (43% protein, 8% lipid, 9% ash, 2% fibre, plus vitamins; 'Aliment Geniteur', Moulin de Saint-Vincent, La Tamoa, New Caledonia, France). The shrimp were kept in the hatchery for 20 days (PL20) before being transferred to the experimental tanks, after which they were considered 'juveniles'.

Sixteen 500 L tanks under an outdoor shelter of 80% shade-cloth with a semi-closed supply of seawater were used for the experiment. Each circular, flat-bottom tank (0.92 m^2), with a central standpipe for water outlet, was supplied with moderate aeration and covered with 80% shade-cloth to further reduce light. The bottom of each tank was covered with a 5 cm layer of rinsed carbonate sand. Sediment samples from five random tanks were bleached, sieved and the grain size fractions weighed following Purcell (1997). Calculation of mean grain size (dry weight) followed the procedures of Dyer (1986).

All tanks were spiked with 3 g of Algamac-2000®(Aquafauna Bio-Marine, Hawthorne, CA, USA) powdered algal food, 3 days before the experiment, to boost the organic content of sediments. Before commencing the experiment, one random 70 mL sediment sample was taken from the upper 25 mm of sediments in each tank for estimating organic content, as described below.

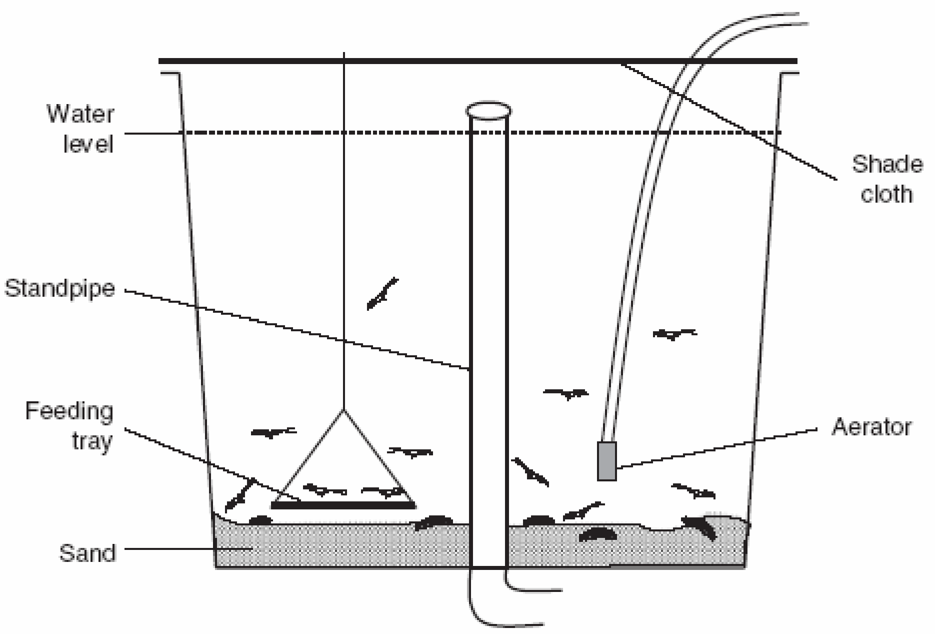


Figure 1: Design of experimental tanks, depicting a replicate of the co-culture treatment. Note that the feeding trays were left in tanks for only 1h, twice daily.

Experimental procedure

A total of 330 juvenile sandfish of 1-4 g (20-36 mm) were firstly isolated in separate tanks without sand for 24 h before the start of the experiment, to allow them to evacuate sand from their guts. Thirty randomly selected juveniles were blotted dry then weighed to the nearest 0.01g, giving a mean weight of 1.7 g (±0.8 g SD). Then 11 groups of 30 juvenile sandfish were prepared for experimental tanks by dispersing individuals of different sizes evenly among the groups, which were then weighed, as a group, in the same manner and randomly assigned to tanks. Juvenile shrimp were counted into groups of 100, which were then strained in a pre-weighed, mesh bottomed container and weighed to the nearest 0.01g, giving a mean group weight of 16.8 g (± 1.7 g SD).

The experimental tanks were randomly assigned one of three treatments: shrimp in monoculture (one replicate tank later cracked and was removed from the experiment; n=4); sandfish and shrimp in co-culture (n=6); sandfish in monoculture (n=5). To supply the formulated shrimp feed (as described above) to the tanks and avoid having uneaten feed contaminate the substrate, feed was sieved to 41mm and placed on feeding trays (40x40 cm) constructed from a wood frame and 1mm mesh. On each morning (ca. 08.30 h) and afternoon (ca. 14.30 h) of the experiment, 2 g portions of wetted feed on the feeding trays were lowered into each tank to 5 cm above the sand surface (Fig. 1). The trays were left for 1h and uneaten feed was then discarded. Thus, shrimp had access to the feed, but sandfish could feed only on the organic content of the sediments and, in co-culture, the faeces of shrimp.

Every 2 days, all tanks received 20% exchange of fresh, sand-filtered seawater. During the first week of the experiment, a deluge of rain from a cyclone lowered salinity from 28-30 to 20 g L^{-1} in the tanks. Sandfish or shrimp that died after this event were removed from the tanks and were not replaced with new animals, as this would confound later comparisons of growth between treatments.

Data collection

On each day, the salinity, O2 concentration, temperature and pH at 20 cm above sediments in tanks were measured between 08:00 and 09:00 hours (using a refractometer and electronic multimeters, respectively, with a precision of 1g L^{-1} salinity, ± 0.2°C, ± 0.3 mg L^{-1} O_2 and ± 0.01 pH).

Before the last water exchange of the experiment, water samples were taken from each tank and passed through a 1 mm particle filter. Nitrite concentration of the samples was determined following Bendschneider and Robinson (1952), with detection by a spectrophotometer (Spectronic Genesys 5, Thermo Electron, Waltham, MA, USA) at 543 nm. Total ammonia-N (i.e. NH_3 –NH_4^+ - nitrogen) concentration was determined following Aminot and Chaussepied (1983), with spectrophotometric detection at 630 nm.

A 100 mm diameter tube with a transparent bottom was used to examine the burrowing frequency of sandfish in tanks. The number of juvenile sandfish with more than half of their body visible on the sand surface at 14:00 hours each day was determined for each tank by lowering the tube to within 15 cm of the sand surface.

A 3-week duration was employed for the experiment because of the availability of the tanks, and because the tanks had to be emptied to recover all sandfish and we wanted to verify a precise period of potential effect of juvenile shrimp or sandfish. In comparison, running the experiment for 4-5 months over the complete culture cycle of shrimp would prevent knowing the developmental stage or time at which potential effect on either species occurred. The duration of the experiment was also set to simply characterize the potential effect of co-culture on the pond environment and on shrimp production during the juvenile phase. After 3 weeks, two sediment samples from the upper 25 mm of sediments were taken randomly within the tanks. The redox potential of the interstitial water was measured to the nearest mV (MultiLine 325; WTW, Weilheim, Germany). Water was then siphoned out of each tank through a mesh screen. Shrimp were collected using nets, whereas sandfish were collected by hand and by rinsing all sediment in the tanks through a 3 mm sieve. The animals were then counted and reweighed as described above; for sandfish, each group was left in a mesh bag in an aerated tank for 24 h overnight to allow defecation of sediments before being re-weighed.

The sediment samples from tanks (from the start and end of the experiment) were oven dried to constant weight at 60 1C. Sub-samples of ca. 20 g were weighed to the nearest 0.1mg then bleached with 10% $NaHClO4$ to remove organic material following Purcell (2000). The sub-samples were reweighed to determine organic content by weight, which avoids biases inherent in loss on ignition methods for determining organic content when carbonate sediments are present (Purcell 1997).

Statistical analyses

Differences in the average individual growth and proportional survival of sandfish and shrimp between monoculture and co-culture were examined using separate one-way ANCOVA tests, with initial group weight and average water temperature in tanks (across days) as covariates. Least-squares linear regression was used to test the relationships between (1) the number of shrimp surviving and their growth and (2) the proportion of surviving sandfish (arcsine transformed) and their growth. Differences in the proportional frequency of burrowing of sandfish between co-culture and monoculture were tested in the same way. Data on proportional survival were arc-sin(y) transformed because of the high range in these data. Differences among treatments in the average pH, concentrations of total ammonia-N and nitrite of water in tanks were tested using one-way ANOVA. Differences in the final organic content of sediments among treatments were tested using one-way ANCOVA, with the organic content at the start of the experiment as the covariate. Levene's test confirmed that variances were homogenous among groups for each test ($P>0.05$). The relationships between growth of sandfish or shrimp and the average ammonia-N and nitrite concentration in tanks were tested with multiple linear regression; data on pH were not included because they appeared to be autocorrelated with ammonia-N concentrations.

Results

Survival

Survival of juvenile shrimp was high, ranging from 79% to 97% among tanks. The survival of shrimp was less variable in monoculture, but was not significantly different between treatments ($P=0.98$) and was neither significantly related to initial group weight nor average water temperature ($P>0.34$). The survival of shrimp did not appear to have a significant effect on their average growth ($P=0.92$).

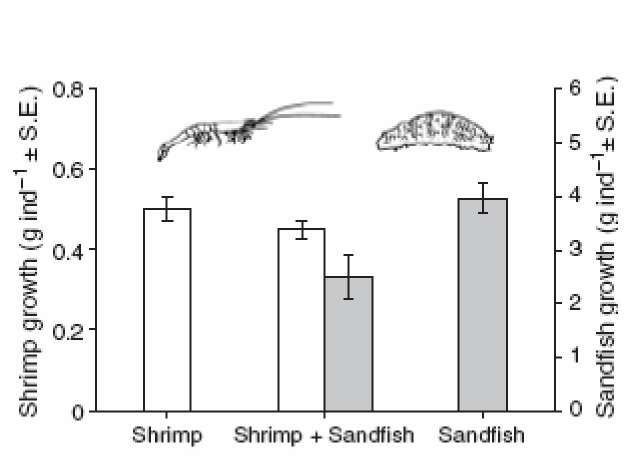
Survival of juvenile sandfish ranged from 73% to 100%. Survival of sandfish in tanks in monoculture varied similarly to the survival in tanks in co-culture and was not significantly different between treatments ($P=50.76$). The covariates, water temperature and initial group weight, did not significantly affect juvenile survival ($P=0.21$ and 0.67 respectively). Deaths of sandfish were most apparent in the days following the drop in salinity after the cyclone. Although the density of individuals in some tanks was then lower, this had no significant effect on growth measured at the end of the experiment ($P=0.62$)

Growth

Shrimp growth over the 3 weeks ranged from 0.39 to 0.59 g individual^{-1} and was equally variable in monoculture and co-culture (Fig. 2). The average individual growth of shrimp was not significantly different between treatments (P=0.19). The average growth of shrimp was not related to their average initial weight (P=0.70), but the effect of water temperature on shrimp growth was marginally significant (P=0.05).

The weight gain of sandfish was much higher per individual and per group than for shrimp over the 3 weeks. The growth of juvenile sandfish in co-culture was significantly lower than in monoculture (Fig. 2; P=0.03), and variation among tanks was similar between treatments. The effect of initial group weight on sandfish growth was marginally non-significant (P=0.07), and water temperature was not significantly related to sandfish growth (P=0.27). However, while growth of sandfish was not related to final nitrite concentration (P=0.45), it was significantly, inversely related to final ammonia-N concentration (β= -0.63; P=0.04).

Figure 2: Average growth over the 3 weeks of shrimp in monoculture (n=4), shrimp and sandfish in co-culture (n=6) and sandfish in monoculture (n=5). Note the change in scale for the two species.



Sandfish behaviour

Juvenile sandfish generally burrowed more frequently in the morning and foraged on the sediment surface later in the afternoon. The average proportion of sandfish visible on the surface at 14:00 hours did not differ significantly (P=0.76) between monoculture (18.9 ± 1.8% SE) and co-culture (18.3 ± 2.5% SE). The variation in average burrowing frequency among tanks was not significantly related to initial group weight or average water temperature (P=0.55 and 0.89 respectively). When the sandfish juveniles were collected at the end of the experiment, most were located within the upper 3 cm of the sediment.

Environmental parameters

Total ammonia-N concentrations differed significantly (P=0.04) between treatments, being higher with shrimp in monoculture and co-culture than in tanks with sandfish alone (Tab.1). Likewise, the average pH differed significantly among tanks (P<0.01), being lower in tanks with shrimp in monoculture and co-culture than in tanks with sandfish alone (Table 1).

Nitrite concentrations were variable among tanks, ranging from 0.04 to 6.47 mg L^{-1}and means did not differ significantly among treatments (P=0.70). Likewise, values for redox potential were variable within and among tanks, ranging from 89 to 189 mV and means were not significantly different among treatments (P=0.79). The average grain size of sediments in tanks was 747 μm (± 227μm SD). The initial organic content of sediments averaged 1.2% (± 0.2% SD), by weight, and declined in most tanks over the experiment. The mean organic content in sediments at the end of the experiment was lower in tanks with sandfish in co-culture and monoculture than in monoculture (n 54), shrimp and sandfish in tanks with shrimp alone (Table 1) but differences were non-significant (P=0.51) and unrelated to initial organic loads (P=0.74).

Table 1: Means (± SE) for biochemical parameters in experimental tanks

Treatment	n	pH	Total ammonia-N (μgL^{-1})	Organic content (% dry weight)
Shrimp	4	8.11 (±0.01)	83(±7)	1.05 (±0.17)
Shrimp1Sandfish	6	8.11 (±0.01)	95(±13)	0.85 (±0.08)
Sandfish	5	8.14 (±0.01)	54(±5)	0.92 (±0.10)

Water parameters of pH and total ammonia-N are from tank averages over the last 2 weeks of the experiment. The organic content of sediments from tanks was at the end of the experiment.

Discussion

The sandfish *H. scabra* can be cultured reliably with the blue shrimp *L. stylirostris* during the early juvenile stages of both species. At the experimental scale, the presence of juvenile sandfish, a deposit feeder that forages on the sediment surface, did not affect the growth or survival of juvenile shrimp in co-culture. The nature of the experiment also suggests that these animals could be grown *en masse* to larger sizes in earthen ponds and is an initial step towards evaluating commercial co-culture. The fact that sandfish grew slower with shrimp shows that monoculture would be favourable for simply on-growing sandfish. However, co-culture with shrimp will be more cost effective for growing sandfish where costs of ponds and their maintenance are high and already met by an existing shrimp culture industry (cf. Jakob et al. 1993).

Litopenaeus stylirostris is normally stocked into ponds around the Pl-20 stage, as in our experiment, but the stocking densities in the tanks were higher than normally used in ponds (100 vs. 20-35 individuals m^{-2} respectively; Martin,Veran, Guelorget & Pham 1998).However, even at high densities, juvenile shrimp grow faster in ponds because natural productivity is more available (Focken, Groth, Coloso & Becker 1998). As such productivity was not available in the tanks, the relatively slow growth of shrimp was not surprising. Additionally, growth was related to average temperatures of tanks, which is common for other shrimp species (Chen, Lin, Chen & Lin 1996; Ponce-Palafox, Martinez-Palacios & Ross 1997). Despite the high densities, shrimp survival (89± 5% SD) was high compared with survival of this species at high density in ponds (ca. 40% over 183 days; Martin et al. 1998). Therefore, the feeding regime and environmental conditions in the tanks can be considered as favourable.

Higher absolute weight gains of sandfish compared with shrimp are noteworthy, particularly as organic loads in sediments were low (~1% by wt) and sandfish did not have access to feed pellets. The mean growth rates for sandfish (0.15 g day^{-1}) were in the order of growth obtained for juvenile sandfish of similar size in tanks when fed on powdered algal feeds (0.1±0.4 g day^{-1}; Battaglene et al.1999). In view of the low organic loads, we conclude that juvenile sandfish were efficient at extracting and assimilating organic matter from the sediments.

Our findings suggest that juvenile shrimp did not disturb the foraging of sandfish. Sandfish juveniles, both wild and hatchery produced, burrow in sediments during the early morning and surface around mid-day to commence foraging on sediments (Mercier, Battaglene & Hamel 1999, 2000). This behaviour is thought to have arisen as a form of protection against diurnal predators (Mercier et al. 1999). Sandfish juveniles that are handled appear to subsequently burrow more frequently (Purcell, Blockmans & Agudo, 2006). If the juvenile blue shrimp were touching or agitating sandfish, we would have expected increased burrowing, but this was not the case.

The slower growth of sandfish in co-culture with blue shrimp appears to have been because of lowered water quality, particularly from increased ammonia levels. Ammonia is the most common toxicant in culture systems (Colt & Armstrong 1981) and can be elevated by direct excretion by shrimp and released through their degraded faeces (Martin et al.1998 and citations therein). The inverse relationship between ammonia concentrations and sandfish growth suggests that soluble nitrogenous wastes from shrimps will limit the grow-out of sandfish in a large-scale co-culture. The ammonia concentration appeared to be the only variate to explain the lower growth of sandfish in co-culture, but growth of shrimp was apparently unaffected.

Many sea cucumber species feed on the upper few millimetres of sediments and eat a range of organic matter, primarily bacteria and non-living detrital matter (Moriarty 1982; Uthicke 1999). Although the sandfish juveniles appeared to be efficient bottom feeders, they did not significantly lower the organic matter of sediments compared with tanks with shrimp alone, so they cannot be expected to be significant bioremediators for shrimp ponds. Notwithstanding, further studies should test the potential bioremediation of pond sediments and waste discharge by larger sandfish at higher densities. Although sandfish do bioturbate sediments by burrowing (Purcell 2004) and may reduce organic wastes by feeding, further studies on these processes should be conducted over several months to allow biochemical interactions to stabilize in ponds.

Our results suggest that sandfish juveniles can be grown to larger sizes in co-culture with juvenile blue shrimp. As sandfish can grow well in earthen ponds (Pitt 2001; Pitt & Duy 2004; Purcell 2004), their co-culture with blue shrimp would solve the major space constraint of growing sandfish juveniles in tanks, giving a feasible mode of mass production to larger sizes for restocking into the wild (Battaglene & Bell, 2006). Alternatively, commercial co-culture of sandfish and shrimp could be synergistic, but the viability will hinge on evidence from further studies that these two species can co-exist at various stages of culture without affecting shrimp production. At least at the juvenile stage of both species, this seems possible.

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