The Atlantic blue crab *Callinectes sapidus* spreading in the Tyrrhenian sea: evidence of an established population in the Stagnone di Marsala (Sicily, southern Italy)

Širenje atlantskog plavog raka Callinectes Sapidus u Tirenskom moru: dokazi o postojanju ustanovljene populaciji u laguni

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DOI 10.17818/NM/2023/SI6
UDK 595.3 (261)*595.3(262)
Preliminary communication / *Prethodno priopćenje*
Paper received / *Rukopis primljen:* 28. 2. 2023.
Paper accepted / *Rukopis prihvaćen:* 19. 5. 2023.

Abstract

The blue crab *Callinectes sapidus* is a portunid brachyuran native of the Atlantic coasts of America. In the last decades, the species has greatly expanded its range in invaded areas, currently including the Atlantic coasts of Morocco, the Mediterranean Sea, the Black Sea as well as the eastern Atlantic coasts of Europe from Portugal to France, Belgium, and Germany. In the present study, several specimens of *C. sapidus* were collected in May, June and July 2021 in the Stagnone di Marsala, a semi-enclosed basin located in the north-western coast of Sicily (southern Italy, Mediterranean Sea) characterized by low hydrodynamics and water exchange with the adjacent open sea and high salinity conditions. Juveniles at different instar stages and ovigerous females were repeatedly captured, suggesting the presence of an established population of the blue crab, despite the peculiar environmental conditions characterizing the basin. These findings are discussed in the context of the current expansion of the species along the African coasts of the Mediterranean Sea.

KEY WORDS

non-indigenous species
Atlantic blue crab
invasive species
Mediterranean Sea
established population
marine coastal area

Sažetak

Plavi rak, Callinectes sapidus je podrijetlom s atlantskih obala Amerike. U posljednjim desetljećima vrsta je uvelike proširila svoj areal u novim područjima, trenutno uključujući atlantske obale Maroka, Sredozemno more, Crno more kao i istočne Atlantske obale Europe, od Portugala do Francuske, Belgije i Njemačke. U ovoj studiji nekoliko je primjeraka C. sapidus sakupljeno u svibnju, lipnju i srpnju 2021. u Stagnone di Marsala, poluzatvorenom bazenu smještenom na sjeverozapadnoj obali Sicilije (južna Italija, Sredozemno more) kojeg karakteriziraju niska hidrodinamika i izmjena vode sa susjednim otvorenim morem i uvjeti visoke slanosti. Mlade jedinke u različitim razvojnim stadijima i ženke s vidljivim jajima više su puta uzorkovani, što sugerira prisutnost reproduktivno ustanovljene populacije plavoga raka, unatoč osebujnim ekološkim uvjetima koji karakteriziraju ovo stanište. O ovim nalazima raspravlja se u kontekstu recentne ekspanzije vrste duž afričkih obala Sredozemnog mora.

KLJUČNE RIJEČI

alohtone vrste
atlantski plavi rak
invazivne vrste
Sredozemno more
ustanovljena populacija
morsko obalno područje

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1. INTRODUCTION

The Atlantic blue crab *Callinectes sapidus* is a portunid brachyuran native of the western Atlantic coasts, with a distribution extending from New Scotia to Uruguay [1]. Itis a euryhaline and eurythermal species, inhabiting transitional systems such as estuaries and lagoons, as well as coastal marine environments where it lives on sandy and muddy bottoms at depths from a few meters up to 90 meters [1, 2]. The life cycle of the species takes place under open sea conditions as well as in transitional environments [2]: adults mate in brackish waters, where males preferentially establish and moult; after mating, ovigerous females move toward open sea environments, where, after hatching, larval stages are released. After returning to brackish environments, juveniles grow rapidly during the first summer and reach maturity in the second year. The carapace width at maturity ranges from 110 to 200 mm for males and 100 to 180 mm for females, and a maximum size of 250 mm can be achieved [2].

C. sapidus has been recorded in Mediterranean waters since the 20th century [3]; it has been recognized as invasive since 2006 [4], yet in the last decade the species has greatly expanded its range, currently including the Atlantic coasts of Morocco, the Mediterranean Sea, the Black Sea as well as the eastern Atlantic coasts of Europe from Portugal to France, Belgium, and Germany [5-7]. In Italian waters, *C. sapidus* is currently recorded throughout the Adriatic Sea, while for the Tyrrhenian Sea and the islands, the number of records is relatively lower (Figure 1).

Recently, a review of records of *C. sapidus* in Italian waters has been performed, reporting two new records in the central Tyrrhenian Sea [8] (Table 1). For the Sicily Island, several occurrences have been recorded and reviewed in [17]; however, data on established populations in this area are still relatively scant. Here, we focus on the Stagnone di Marsala (north-western coast of Sicily) where the presence of *C. sapidus* was firstly recorded in an environmental impact assessment report in 2015 [18] and was subsequently confirmed in June 2020 [17]. However, both records did not include additional quantitative information, and, in particular, did not provide confirmation of the actual establishment of the species in the area, as it is testified by the occurrence of e.g., ovigerous females or different ontogenetic stages [19-21]. Furthermore, no additional information is available regarding the presence of the species in the Tyrrhenian sector of the Sicily Island, with the exception of a single individual captured in 2020 on the coast of Favignana Island in the Egadi Archipelago located about 10 km far from the Stagnone di Marsala [22]. In the present study, data on the repeated capture of ovigerous females and juveniles of *C. sapidus* in the Stagnone di Marsala are reported for the first time, ultimately suggesting the presence of an established, reproductively active population in the area.

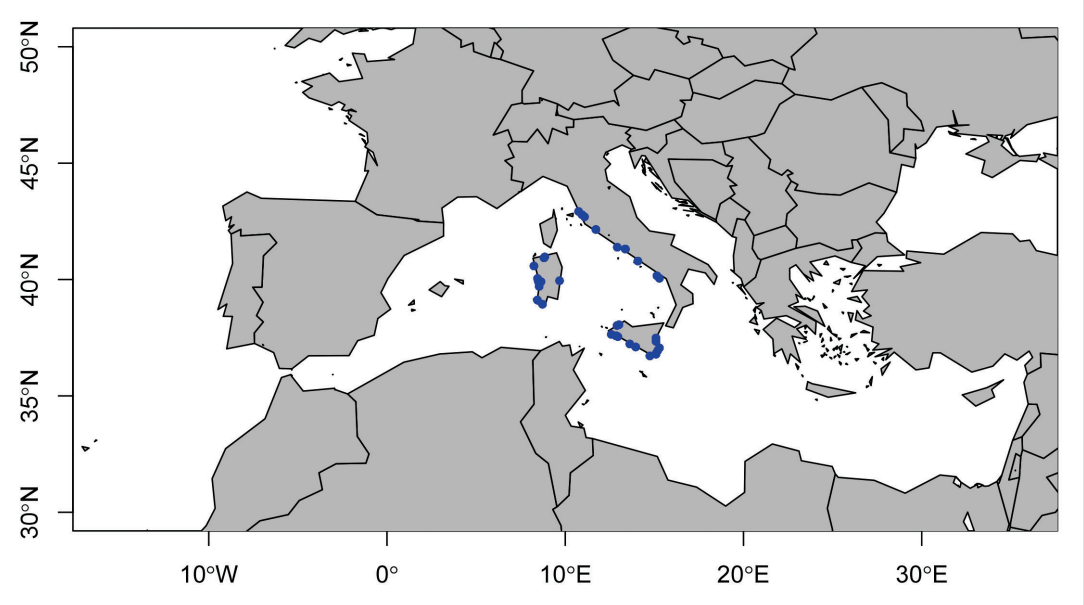

Figure 1 Distribution of the Atlantic blue crab, *Callinectes sapidus* in the Tyrrhenian Sea, Sicily and Sardinia. Occurrence refers to records reported in Table 1

Table 1 List of reports of the Atlantic blue crab, *Callinectes sapidus* in the Tyrrhenian Sea, Sicily and Sardinia

Region	Location	Latitude	Longitude	Year	Habitat	n. individuals	Collection	References
Campania	Miseno (NA)	40.7930°N	14.0764°E	2019	Coastal lagoon	1	Photographic report	[8]
	Palinuro (SA)	40.0528°N	15.2825°E	2019	Coastal zone	5-10	Collected specimens	[9]
Latium	Civitavecchia (RM)	42.1485°N	11.7205°E	2019	Estuary	1	Trammel net	[8]
	Rio Chiaro (LT)	41.309673°N	13.377748°E	2020/2021	Inland waters	1	Fyke net	[10]
	Lago di Fondi (LT)	41.312694°N	13.363083°E	2020/2021	Inland waters	1	Fyke net	[10]
Sardinia	Cabras (OR)	39.9101°N	8.49245°E	2018	Pond	3	Fyke net	[11]
	Calich (SS)	40.932°N	8.80800°E	2018	River	14	Fish trap	[11]
	Coghinas (OR)	40.933°N	8.810°E	2017	Pond	14	Fishtrap/gillnet	[11]
	Fenosu (OR)	39.9094°N	8.64202°E	2017	Pond	1	Fish trap	[11]
	Is Bena (OR)	40.0437°N	8.44578°E	2018	Pond	1	Fish trap	[11]
	Lazzaretto beach (SS)	40.580°N	8.245°E	2018	Coastal zone	1	Recreational	[11]
	Matzaccara (CI)	39.1180°N	8.43805°E	2018		1	Funnel trap	[12]
	Merceddì (OR)	39.705°N	8.538°E	2018	Pond	1	Fish trap	[11]
	S'Ena Arrubia (OR)	39.8289°N	8.55364°E	2017	Pond	61	Lavoriero	[11]
	Tirso (OR)	39.9098°N	8.54996 E	2017	River	1	Fish trap	[11]
	Balestrate (PA)	38.0573°N	13.01121°E	2019	Coastal zone	4	Trammel net	[13]
Sicily	Capo Peloro (ME)	38.259782°N	15.628214°E	2019	Lagoon	1	Handily collected	[14]
	Castellammare del Golfo (TP)	38.0250°N	12.9074°E	2019	Estuary	2	Rod fishing	[9]
	Catania (CT)	37.4867°N	15.0891°E	2019	Estuary	6	Visual report	[9]
	Contrada Fiori Sud (AG)	37.5469°N	12.9514°E	2019	Coastal zone	2	Visual report	[9]
	Licata (AG)	37.101367°N	13.952483°E	2016	Coastal zone		Trammel net	[15]
	Mazara del Vallo (TP)	37.642°N	12.58400°E	2018		5	Trammel net	[16]
	Sampieri (RG)	36.7172°N	14.7479°E	2018	Coastal zone	1	Trammel net	[9]
	Valle del Belice (TP)	37.5819°N	12.8653°E	2016	Estuary	2	Trammel net	[9]
	Vendicari (SR)	36.7897°N	15.09°E	2018	Coastal lagoon	3	Collected simple	[9]
	Villaggio San Leonardo (SR)	37.3439°N	15.0925°E	2018	Estuary	20	Trammel net	[9]
	Villa Croia (GR)	42.784419°N	10.940617°E	2020/2021	Inland waters	1	Fyke net	[10]
Tuscany	Spergolaia (GR)	42.694778°N	11.081806°E	2020/2021	Inland waters	1	Fyke net	[10]

2. MATERIALS AND METHODS

2.1. Study site

The Stagnone di Marsala is a semi-enclosed basin with a surface area of 20 km^2, located in the north-western coast of Sicily (37.860463° N; 12.462289° E). This lagoon-like basin is characterized by shallow waters with an average depth of 1.5 m and two openings towards the sea: Bocca San Teodoro (400 m wide, 0.3 to 0.4 m deep) in the northern sector and Bocca Grande in the south (1200 m wide, 1.0 to 2.0 m deep) [23]. The basin is characterized by low hydrodynamics and limited water exchange with the adjacent open sea; consequently, high salinity and wide temperature range are generally observed [24,25]. No freshwater inputs are present inside the Stagnone [26], while about 1.5 km north of the Bocca San Teodoro an ephemeral freshwater source is represented by the mouth of the Birgi River, which is barred in summer. Despite its proximity, this freshwater input does not influence the salinity of the Stagnone di Marsala, which indeed is a hyperhaline basin. The basin is generally characterized by sandy-muddy bottoms densely colonised by the seagrass *Cymodocea nodosa* often mixed with *Caulerpa prolifera*, while patchy *Posidonia oceanica* beds occurs in the central part of the basin [23].

2.2. Sample collection and processing

On May 27th, June 29th and July 13th 2021 sampling operations were carried out in five sites along the north-south axis of the Stagnone di Marsala (site *a*: 37.905572° N, 12.457480° E; site *b*: 37.886250° N, 12.470067° E; site *c*: 37.874350° N, 12.484617° E; site *d*: 37.843978°N, 12.464438°E; site *e*: 37.814583° N, 12.457250° E) (Figure 2).

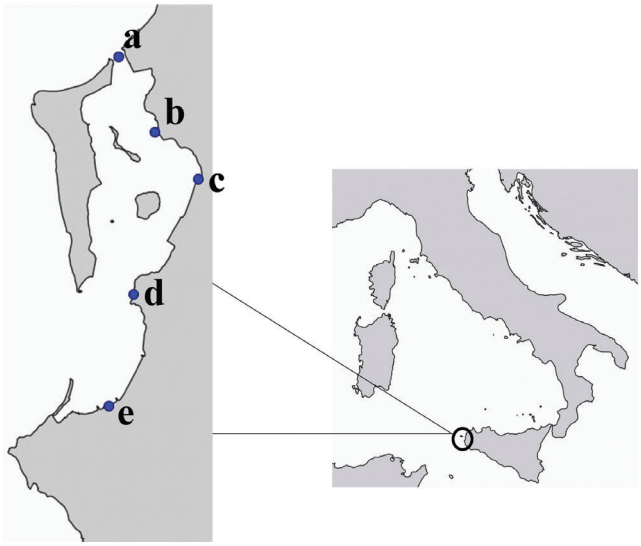

Figure 2 Sampling sites within the Stagnone di Marsala, Sicily, Italy

Crabs were captured using baited traps (60 x 30 cm) deployed between 6.00 p.m. and 9.00 p.m. at each site and date and retrieved the day after between 5.00 a.m. and 10.00 a.m. and hand-held fish nets (1.5 x 4 m) with a mesh size of 2 mm. At each site, before deploying the traps, water temperature and salinity were measured with a multiparametric probe (Hanna® HI98194). Captured specimens were put in individual plastic bags and transferred to the laboratory in refrigerated containers. In the laboratory, crabs were identified on the base of the presence of two obtuse and large teeth on the front between the inner orbital teeth [27]. Afterwards, specimens were wet weighed to the nearest 0.1 g using a digital balance and had their carapace length (CL) and the carapace width (CW) measured to the nearest mm using a calliper. Ovigerous females had the eggs removed before determining the individual wet weight. Carapace width was determined as the distance between the two lateral spines, and the carapace length was measured from the teeth of frontal margin to the end of dorsal carapace. Furthermore, ontogenetic stage and sex were determined for each specimen by inspection of the abdomen shape. The egg masses removed from ovigerous females were wet weighed; subsequently, eggs were counted using a stereomicroscope in a pre-weighed subsample. The total number of eggs per female was estimated from the total weight of egg masses.

3. RESULTS AND DISCUSSION

In total, twenty-two specimens were captured, five males and seventeen females;19 individuals were adults and only three juveniles (Table 2). Among adults, three were males and sixteen females of which three were ovigerous (Table 3). All juveniles were trapped in the southern part of the Stagnone di Marsala, at site *d*, where the average salinity and temperature were 42.20 psu and 27.49 °C respectively (Table 4). The first was a male 5th- 7th instar (~ 20 mm CW) (Figure 3), sampled on May 27th. Afterwards, a male and a female 11th -15th instar (~ 70-100 mm CW) were caught on July 13th. Adult males were caught only in the southern zone at sites *d* and *e*, in two out of three sampling occasions (Table 4). Females (Figure 4) were captured at every sampling occasion, in both the northern and southern areas of the Stagnone di Marsala, where salinity averaged 39.78 psu and 41.14 psu and temperature averaged 27.84 °C and 28.51 °C respectively (Table 4). Specifically, eight adult females were collected at site *a*, and eight were captured at site *e*.

Figure 3 Juvenile sampled at site *d*; A) dorsal view and B) ventral view

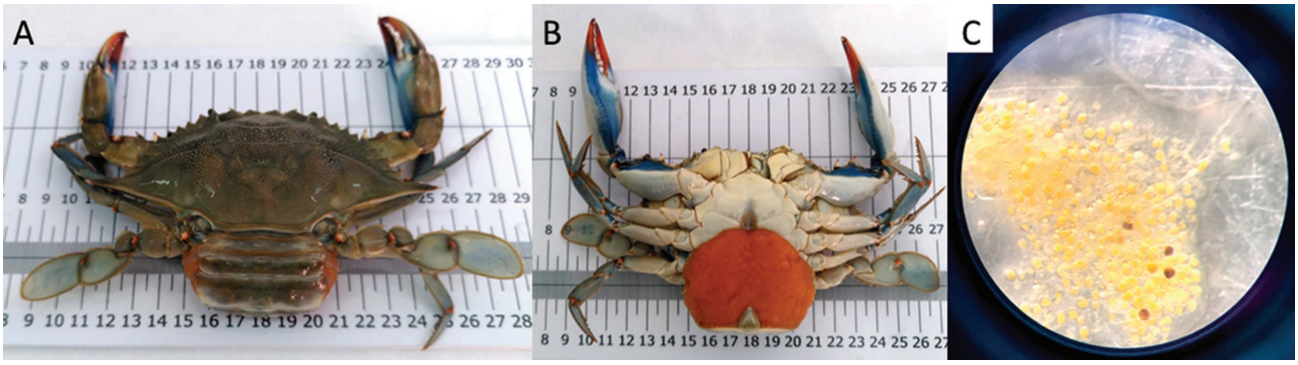

Figure 4 Ovigerous females sampled at site *a*; A) dorsal view, B) ventral view and C) eggs at microscope

Table 2 Number of specimens per sampling date and life stage; for each sampling, the ranges of carapace width (CW), carapace length (CL), and wet weight (WW) are reported

Sampling date	site	N. males (CW>100mm)	N. females (CW>100 mm)	N. males (CW<100 mm)	N. females (CW<100 mm)	CW mm ranges	CL mm ranges	WW g ranges
27/05/2021	*a*	0	**1**	0	0	135.19	65.62	127.85
	b	0	0	0	0	\	\	\
	c	0	0	0	0	\	\	\
	d	0	0	**1**	0	33.80	19.40	4.10
	e	**2**	**1**	0	0	114.20-153.41	56.82-72.24	108.24-196.79
29/06/2021	*a*	0	**1**	0	0	149.50	71.40	199.28
	b	0	0	0	0	\	\	\
	c	0	0	0	0	\	\	\
	d	0	0	0	0	\	\	\
	e	**1**	**1**	0	0	135.64-139.62	66.06-68.28	161.80-201.38
13/07/2021	*a*	0	**7**	0	0	103.89-143.06	50.19-68.48	60.79-166.42
	b	0	0	0	0	\	\	\
	c	0	0	0	0	\	\	\
	d	0	0	**1**	**1**	73.58-94.94	38.94-49.84	34.06-69.45
	e	0	**5**	0	0	120.78-164.05	61.01-78.14	107.23-234.96

N, number; CW, carapace width; CL, carapace length; WW, wet weight

Table 3 Biometric data of ovigerous females (carapace width, CW; carapace length, CL; wet weight, WW) and estimated weight and number of eggs

date	site	cw (mm)	Cl (mm)	ww (g)	total eggs weight	estimate egg number
13/07/2021	*a*	119.5	58	104.4	18.9	1,587,742
13/07/2021	*a*	134.6	66.7	145.7	26.2	1,853,617
13/07/2021	*e*	134.3	65.3	140.4	37.46	2,890,440

CW, carapace width; CL, carapace length; WW, weight wet

Table 4 Temperature and salinity in different sites and sampling dates

site	27/05/2021		29/06/2021		13/07/2021	
	Temperature (°C)	Salinity (PSU)	Temperature (°C)	Salinity (PSU)	Temperature (°C)	Salinity (PSU)
a	30.80	40.40	25.82	39.13	26.90	39.83
b	25.10	46.60	32.07	50.45	28.06	52.22
c	23.70	46.20	27.05	49.38	28.32	51.40
d	22.74	41.00	30.61	43.06	29.12	42.55
e	25.28	39.95	29.13	41.76	31.12	41.72

Interestingly, all *C. sapidus* specimens were collected in either the north or south areas (sites *a*, *d* and *e*), while none was observed in the centre of the lagoon, the area less influenced by the open-sea. Juveniles and males were captured in the southern area, while females were equally collected at the northern and southern areas. Noteworthy, the presence of adult individuals of both sexes, ovigerous females, and juveniles at different stages of development suggests for the first time the occurrence of a stable population in the Tyrrhenian Sea and in Italian Islands. Indeed, the records reported in Table 1 mainly refer to sightings and captures of adult specimens (mainly females) made episodically by fishermen. Thereby, information on the establishment of the blue crab in the Tyrrhenian area remain incomplete. Furthermore, despite the Stagnone di Marsala is a hypersaline basin, even settled juveniles and adult males are present, confirming the ecological opportunism of this species and its tolerance to extreme salinity condition. Along the Atlantic coast of America, this species is generally present under brackish conditions, although there is a hypersaline lagoon in Texas, Laguna Madre, where this species is present [28]. So far, we know that *C. sapidus* invaded earlier the northern and eastern Mediterranean Sea, particularly the Adriatic Sea, Aegean Sea and Levantine Basin [7, 29]. Overall, these invaded areas have environmental features consistent with those characterising native habitats such as Chesapeake Bay, namely those peculiar of brackish transitional waters. Under these salinity conditions, juveniles can settle and grow up and the adults can mate, before the spawning females move toward rivers and lagoon mouths and higher salinity areas to release larvae [2]. Interestingly, at present *C. sapidus* is spreading toward Tyrrhenian Sea and the northern African coasts, invading environments characterised by abiotic conditions far more extreme, especially in terms of salinity, than those generally observed in native habitats [6, 30-33]. The present study in the Stagnone di Marsala

suggests that the species is able to complete its life cycle not only in transitional waters but also under fully marine and hypersaline conditions, opening up interesting future prospects by expanding the range of environments that this species is able to colonize. It is worth to emphasize that additional investigations performed on a year- or multi-year time scale are needed to corroborate the actual establishment of the species in the basin, and to provide more resolved biological information in terms of e.g., size and ontogenetic structure or occurrence of larval stages [19-21]. Considering the peculiar abiotic features of the study area, in particular the wide seasonal variation in salinity and temperature, these studies will provide a more comprehensive picture of the factors driving the spatial distribution of the species. Accordingly, further analyses are ongoing to investigate the population dynamics of *C. sapidus* as well to assess its impact on native vertebrate and invertebrate species. Given its size, *C. sapidus* represents the largest brachyuran - either native and non-indigenous - currently characterizing Mediterranean transitional waters [34]. In-depth assessments of its actual ecological impacts on recipient communities are still scant (but see [35-36]); thus, it might be valuable to study, together with the adaptation strategies of the blue crab in peculiar environments such as the Stagnone di Marsala, the interaction and the possible competition with autochthonous species at different trophic levels. Beyond the negative impact this species may have on invaded community and biodiversity levels, blue crab is a valuable fishery resource in America and in recent years it is becoming a species of interest for fishery also in the Mediterranean Sea [29, 37-38-39]. The exploitation of this valuable shellfish resource is important to mitigate the expansion of this species in invaded areas, with positive economic implications [29, 37-38-39]. Knowing in detail the ecological role of *C. sapidus* allows us to better manage its invasion and limit the consequences that it may have on local ecosystems with socio-economic repercussions (i.e., fishery).

Author contributions: Conceptualization, G. M., R. B., and S. V.; Investigation, R. B. and S. V.; Writing – Original Draft Preparation, R. B.; Writing – Review & Editing, R. B., G. M., S. V. and A. M.; Supervision, G. M., S. V. and A. M.; Project Administration, S. V.; Funding Acquisition, S. V.

Funding: This work was supported by PRIN project "Exploring the fate of Mediterranean microplastics: from distribution pathways to biological effects (EMME)" funded by the Italian Ministry of University."

Conflict of interest: The authors state: "Declarations of interest: none".

Acknowledgments: The authors express their warmest thanks to Laura Ciriminna, Laura Caviglia and Dr. Cecilia Tramati for the essential help during sampling and laboratory work. Moreover, they are grateful to the Libero Consorzio Comunale di Trapani (TP) for the permit to work on field and perform scientific research activities inside the "Stagnone di Marsala Orientated Reserve".

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